Circadian and Homeostatic Sleep-Wake Regulation in Women:

Effects of Age and Major Depressive Disorder

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Contents

Summary

Everyday life of all organisms is governed by rhythmic physiological changes throughout the 24-h day. The most obvious circadian rhythm is the sleep-wake cycle. During a human lifespan the sleep-wake cycle undergoes a number of specific changes, which involve sleep duration, sleep architecture, electrophysiological characteristics, and sleep timing. Age, gender and developmental stage during childhood and adolescence have strong influences on sleep. Abnormalities in sleep-wake cycle regulation are often sequelae of different disorders and may often be the cause of specific diseases. Two groups where marked alterations in sleep-wake cycles occur include adolescents and people with psychiatric disorders. Thereby, women develop sleep disorders such as insomnia as early as during adolescence and they are at much higher risk to develop mood disorders compared to men.

In this thesis we aimed at elucidating homeostatic and circadian aspects of sleep-wake regulation in women with respect to sleep phase preference during adolescence and its association with developmental stage and homeostatic and circadian sleep-wake regulation in major depression.

The first part of this thesis covers an ambulatory study based on a cross-sectional chronotype survey among 1'187 females aged 5 to 51 years. We investigated the influence of age on sleep phase preference as well as its relationship to the onset of menarche, which served as physiological maturation marker. The study results as presented in chapter 2 of this thesis confirm previous findings of age-dependent changes in sleep-wake behaviour as measured by chronotype changes. We found evidence for a new biological marker for the end of adolescence since our data point towards an abrupt change in the delayed sleep phase preference in women 5 years after the onset of menarche towards advancing the sleep-wake cycle. This heralds the beginning of *adult-like* sleep-wake behaviour in women. We found strong evidence for a circadian misalignment in adolescents as they experience a so-called *social jet-lag* between week and free days accounting for up to 3 hours at the nadir of the delayed sleep phase preference 5 years after menarche. This result is of particular importance since circadian misalignment of the sleep-wake timing and the circadian pacemaker may lead to impaired alertness and performance during wakefulness as well as to sleep disorders and depression.

In the second part of this thesis (chapters 3 and 4) we compared homeostatic and circadian aspects of sleep-wake regulation in young women suffering from a major depressive disorder with age-matched young healthy women and healthy older women under low and high sleep

pressure under stringently controlled laboratory conditions (constant routine conditions). The study design comprised two study protocols starting with a 8-h baseline night and ending up with a 8-h recovery night. The time between these two nights (40 hours) either consisted of sustained wakefulness (SW protocol; high sleep pressure condition) or 10 short sleep-wake cycles with alternating episodes of 75 min of sleep and 150 min of wakefulness (nap protocol; low sleep pressure condition). We investigated the sleep electroencephalogram (EEG; 0.75-25 Hz) during all scheduled sleep episodes and the homeostatic sleep response to enhanced and reduced sleep pressure by EEG slow-wave activity (SWA; spectral power in the 0.75-4.5 Hz range) during the recovery nights. Sleep analysis of the 10 nap episodes allowed to compare circadian modulation of the EEG spectra between the three groups as scheduled sleep episodes occurred at different circadian phase while constant low sleep pressure was controlled for.

The homeostatic sleep pressure was overexpressed in young depressed women compared to both healthy control groups under high sleep pressure (chapter 3) as well as under low sleep pressure (chapter 4) as indexed by significantly higher frontal EEG SWA during baseline and recovery nights as well as during some nap episodes. This result was endorsed by significantly enhanced subjective sleepiness in young depressed volunteers under low sleep pressure conditions and higher EEG SWA during the diurnal nap sleep episodes. Enhanced homeostatic sleep drive irrespective of the underlying sleep-wake cycle manipulation implies a trait-dependency of the homeostatic sleep-wake regulation in depression.

Our data gave evidence for an alternated ultradian modulation of EEG SWA in young depressed and healthy older compared to healthy young women. This was reflected by a temporally enhanced SWA rebound during the recovery night under high sleep pressure (chapter 3) and by a non-existent SWA intra-sleep rebound during the recovery night under low sleep conditions (chapter 4).

A reduced melatonin amplitude in the depressed women compared to the healthy young volunteers was observed which implies a weaker signal output of the circadian pacemaker in depression. This evidence was substantiated by the occurrence of more EEG SWA during diurnal naps in depressed volunteers.

Taken together, this thesis provides several insights into circadian and homeostatic aspects of the sleep-wake cycle in women during maturation and in depression. We could establish an association between changes in circadian sleep phase preference during female adolescence and physiological maturation and gained insights in age-dependent female sleep-wake behaviour. Our findings have implications on possible actions in order to prevent *social jet-lag* in adolescents as such that a temporal delay in school start times should be equally dynamic as the sleep phase timing developments in adolescents. Our results on sleep-wake regulation in depression revealed higher levels of SWA in frontal brain areas together with an *over-steering* of the homeostatic response to alterations in sleep pressure levels together

with a weakening of the circadian signal output. We could thus emphasize the misbalance of the opponent interaction between circadian and homeostatic sleep regulation in young depressed women without major sleep disturbances, which may also have repercussions on the treatment of the illness in this endophenotype of depression. Therefore, selective slow-wave sleep and SWA deprivation and bright light therapy could lead to a readjustment of homeostatic and circadian sleep-wake processes and to mood improvement.

1 Introduction

«The best of rest is sleep.»

William Shakespeare, Measure for Measure, Act 3, Scene 1

Sleep inspired the ancient Greeks and Romans. Hypnos ('Ὕπνος') was the god of sleep in the Greek mythology, Somnus its Roman equivalent. Given the fact, that in mammals sleep is coupled with a state of unconsciousness it is not surprising that the ancients perceived Hypnos as the twin of the god of non-violent death (Thanatos; 'Θάνατος') and as the father or the brother of dreams (Oneiroi, 'Ὀνειροι') (THEOI Greek Mythology, www.theoi.com). Over centuries, sleep has been an important topic in philosophy, theology, literature, and art. In contrast to the long lasting perception that sleep is just a suspension of activity we now know that sleep is a very complex rhythmic succession of different physiological, neurological, and behavioural states. This knowledge is based on the work of many pioneers in sleep and circadian rhythm research in particular during the last century. Given the importance of their work some of the milestones are shortly mentioned below.

In 1846 probably the first book solely devoted to sleep was published by Edward Binns [1]. Although it is not based on sound scientific basis it gives some interesting insights into the perception of sleep and its repercussions on mood and alertness when disturbed some of which have been scientifically elucidated more than 100 years later.

> «Sleep is the art of escaping reflection. In animals, it is the cessation of the functions of the voluntary muscles, through the repose of the senses. Sound, or heavy, or dull sleep, is not always an indication of health, for the apoplectic, and some aged persons, sleep much; and hydrocephalic patients, frequently. Yet, they are far removed from the condition of true health.
>
> Narcotic sleep is produced by destroying sensibility; whereas, true sleep is the natural repose of the nervous system. From the one, we awake clouded and confused; from the other, we arise gladdened and refreshed. By the latter, the spirits are rendered buoyant and the mind cheerful; from the former, we experience depression, melancholy, and a dread of some unknown evil.»
>
> Edward Binns, 1846, pp 1-2

But it was only at the beginning of the 20th century when sleep research gained a more physiological perspective with the work of Henry Piéron, a French scientist, who was the first to declare a neurochemical hypothesis on the regulation of sleep based on physiological brain experiments [2]. Sleep research experienced a break through when Hans Berger, a German psychiatrist, discovered the electroencephalogram (EEG) in 1929 and as a result the basic electrical brain activity during sleep and wake in humans were examined which were until then like a *black box*. At the same time, Nathaniel Kleitman, also called the *father* of modern sleep research, started to study the regulation and physiology of sleep and wakefulness in the 1920s [3]. He was one of the main contributors to the modern understanding that sleep is characterised by various dynamic brain processes rather than just a state of quietness as commonly believed earlier. Furthermore, his studies on the endogenous circadian clock in humans and its influence on the sleep-wake cycle in the absence of environmental time cues in so-called cave experiments represent a milestone in human circadian research. Another important contribution to circadian rhythm research during the first half of the 20th century was made by Erwin Bünning, a german botanist, who focussed on photoperiodism and the influence of light on the physiology of plants. He based his research on experiments conducted by the French astronomer Jean-Jacques d'Ortous de Mairan, who was the first person who demonstrated as early as 1720, that daily rhythms evolve endogenously. In the 1950s Colin Pittendrigh together with Jürgen Aschoff and Franz Halberg, also called the founders of circadian physiology, began to systematically study the function of biological clocks and rhythms in humans and animals [4]. Inspired by Kleitman, Aschoff together with Rutger Wever conducted various important bunker experiments on the circadian regulation of the human sleep-wake cycle in the legendary *Andechs bunker*, a chronobiological research lab built into a hill. They showed that the human sleep-wake cycle persists with a near 24-h period in the absence of external time cues, so-called *Zeitgebers* such as light and clock time [5]. In 1953 Aserinsky and Kleitman revolved the knowledge of sleep architecture by the discovery of rapid eye movement (REM) sleep [6] leading to the modern sleep classification scheme, which comprises 5 sleep stages and is still in use today. Until their discovery REM stages were believed to represent awakenings characterised by fast, low amplitude EEG voltage [7]. Soon after the discovery of REM sleep, the cyclic nature of the non-rapid eye movement (NREM) and REM sleep stages in humans and other mammals as well as the approximate duration of the human sleep cycle of 90-100 minutes were described by William Dement who also established the connection between dreaming and REM sleep together with Nathaniel Kleitman [8, 9]. More than thirty years ago the first questionnaires to assess sleep time preference were developed [10, 11] and together with questionnaires to screen for sleep disorders and to assess sleep quality such as the Epworth sleepiness scale and the Pittsburgh Sleep Quality Index for example [12, 13] they allow to gather important insights into the sleep-wake cycle and circadian preferences of study volunteers and patients.

Approximately one third of our live is dedicated to sleep (i.e. on average 8 hours out of each 24 hour day). Although there are many theories on the function of sleep such as metabolic, immunologic, mental, neural repair and reorganization as well as memory processing after

several decades of sleep research it is still not known why we sleep [14, 15].

> «As far as I know, the only reason we need to sleep that is really, really solid is because we get sleepy.»
>
> William C. Dement, pioneer in sleep research, cited from *The Secrets of Sleep*, National Geographic Magazine, May 2010

However, many studies have shown that the amount of sleep and its quality are crucial for many physiological, psychological and cognitive processes. Long-term sleep disturbances can lead to physiological, cognitive, mental, and emotional dysfunctions [16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26]. Moreover, many psychological illnesses such as major depression are often accompanied by biological rhythm abnormalities resulting in sleep disorders. In the following introductory sections some background information will be given on neurobiological aspects of sleep, sleep regulation and its timing. In addition, an overview of the current knowledge about the neurobiology of major depression, in particular with reference to circadian and homeostatic sleep regulation is included.

1.1 Sleep

1.1.1 Healthy sleep profile and electroencephalographic characteristics of sleep

The analysis of the different sleep stages and other characteristics of sleep are based on polysomnographic recordings which include electrical brain waves (EEG) (see Fig. 1.1.1), eye movements (EOG), and skeletal muscle activity (EMG). The first definition of sleep stages by Loomis and colleagues in the 1930s was established on four different stages for sleep and one for wakefulness, which was revolved after the discovery of REM sleep in the 1950s [6, 27]. Since then sleep in humans is described by two main types of sleep: non-rapid eye movement (NREM) sleep and REM sleep. The physiological and neurological manifestation of these two types is rather different, and thus different brain areas are suggested to be responsible for their regulation (see also sections below) [28]. Since 1968 sleep EEG staging criteria are standardized and according to this so-called R&K standard NREM sleep is divided into sleep stages 1-4 with slow-wave sleep (SWS), also called delta sleep, comprising sleep stages 3 and 4 [29] (Fig. 1.1.1). A revised R&K standard was published in 2007 by the American Academy of Sleep Medicine (AASM) which includes several changes, most significantly thereby the combination of stages 3 and 4 into stage N3 [30].

As shown in figure 1.1.1 wakefulness is dominated by alpha waves in the frequency range 8-13 Hz. During NREM sleep stage 1 a transition to slower theta waves (4-7 Hz) occurs which marks the onset of sleep. Stage 1 sleep is very shallow but already accompanied with the loss of most conscious awareness of the external environment and is sometimes also referred to as drowsy sleep.

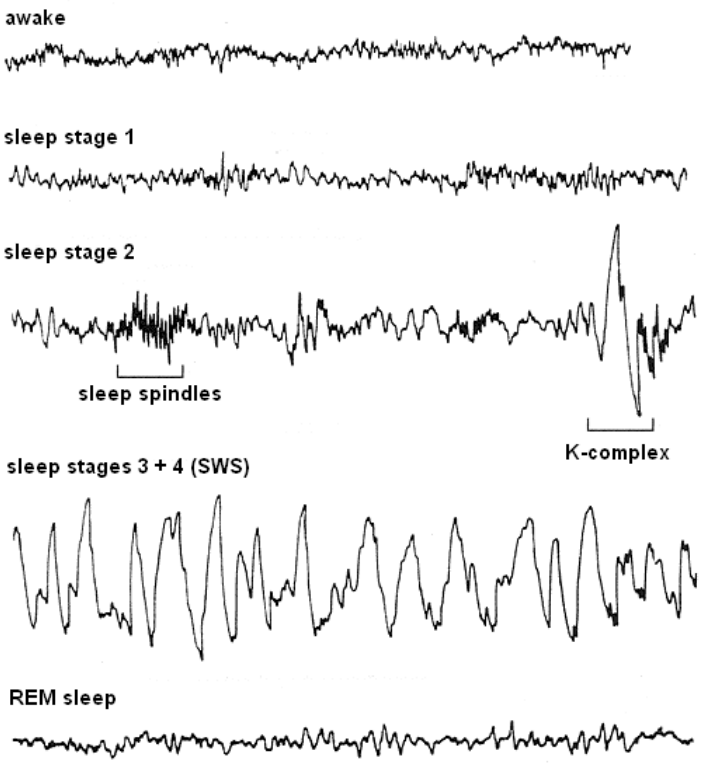

Figure 1.1.1: Typical EEG recordings during different sleep stages and wakefulness

Wakefulness as well as rapid eye movement (REM) sleep are characterized by fast, low voltage EEG but in contrast to the wake stage, during REM sleep muscular activity is very low. The transition from light sleep (stage 1) to deep sleep stages 3 and 4 is accompanied by a general decrease of EEG oscillations (frequency) and an increase of the EEG wave amplitude. Sleep stage 2 occurs for 45-55% of the total time of a night sleep episode and it is characterized by the occurrence of peculiar waves such as sleep spindles and K-complexes.

NREM stage 2 sleep is characterized by distinct electrical brain wave features: sleep spindles ranging from 11–16 Hz (most commonly 12–14 Hz) and K-complexes. Conscious awareness of the environment is obsolete and muscular activity decreased compared to the awake stage. About 45-55% of the total adult night sleep time is occupied by stage 2. Slow-wave sleep (SWS; also called delta sleep or deep sleep) comprising NREM sleep stages 3 and 4 is characterized by a slow high-voltage EEG and the presence of a minimum of 20% of delta waves ranging from 0.75–4.5 Hz (stage 3 = 20-50% delta waves; stage 4 > 50% delta waves) with a peak-to-peak amplitude >75 µV. The occurrence of various parasominias is associated with slow-wave sleep such as sleepwalking and sleep terror. REM sleep is characterized by rapid eye movements as shown by the electrooculogram as well as a waking stage-like rapid low-voltage EEG. During REM sleep muscular tonicity is very low. Most memorable dreams occur during this sleep stage although dreams may also be present during NREM sleep. REM sleep accounts for 20–25% of total adult night sleep time.[31, 32]

Sleep is an ultradian process and its architecture is characterized by sequences of 4-5 NREM-REM cycles (Fig. 1.1.2) whereby each sleep cycle lasts for approximately 90-100 minutes

[33, 34, 35]. In adults sleep is usually entered through NREM which then progresses from shallow to deep stages. This transition is accompanied by characteristic changes in brain rhythms and increased levels of arousal thresholds which reflect an increase in neuronal hyperpolarization and synchronization expressed as sleep spindles and slow-waves (Fig. 1.1.1). During NREM sleep heart rate as well as respiration slow down. REM sleep occurs after NREM stage 4 sleep and apart from the afore mentioned skeletal muscle paralysis and EEG changes irregular patterns of respiration and heart rate are characteristic.

Sleep quality, i.e. feeling relaxed and restored after sleep, depends thereby not only on the total amount of sleep but also on the temporal pattern of the different sleep stages as well as on their duration per cycle since each sleep stage may have a distinct physiological function. Furthermore, the duration and deepness of sleep inertia depends on the sleep stage out of which awakening occurs [36, 37]. The amount of SWS (sleep stages 3 and 4) is highest during the first NREM-REM cycle and decreases thereafter while stage 2 and REM sleep show the opposite pattern in normal healthy sleep. Healthy aging is characterized by a general decrease in SWS, but the general temporal pattern of SWS across the night with initial high SWS values and lower values towards the end of the sleep episode is restored in healthy aging [38].

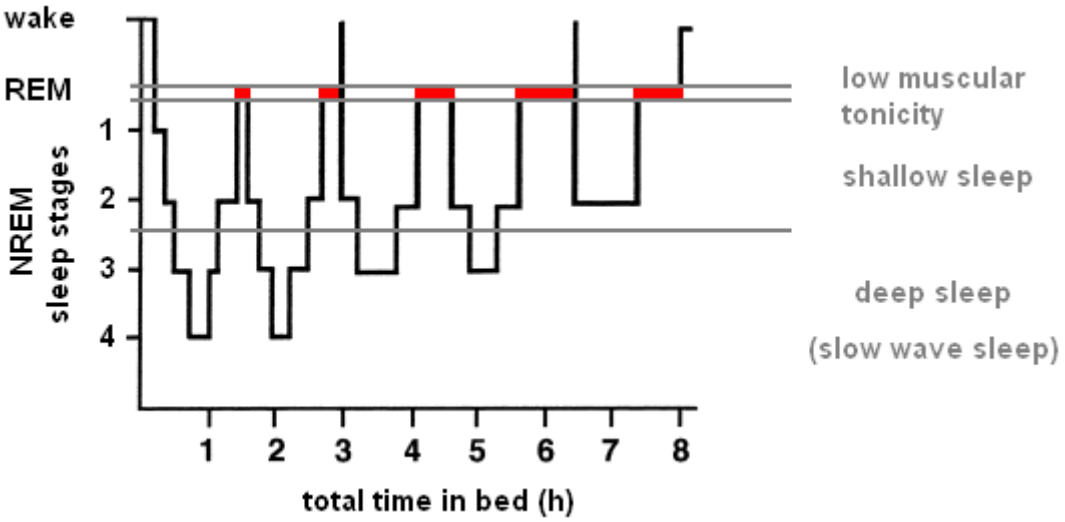

Figure 1.1.2: Hypnogram (sleep profile) of a night sleep episode of a healthy young person

A typical night of sleep consists of 4-5 sleep cycles lasting about 90-100 minutes each. Each cycle is characterized by the occurrence of NREM sleep stages 1-4 and REM sleep (highlighted in red). Towards the end of the night REM episodes tend to be longer. In contrast, the proportion of slow-wave sleep (SWS = sleep stage 3 + 4) is predominant during the first two sleep cycles and diminishes consecutively with advancing sleeping hours. The awakening in the morning ideally (naturally) occurs after REM sleep.

The quantitative assessment of the EEG on the basis of sleep stages derived from visual scoring is rather limited and cannot account for the relative contributions of the single EEG frequencies to the recorded sleep signal. Hence, a transformation of the EEG wave by means

of a fast Fourier transform (FFT) is commonly used to quantify the spectral composition of the EEG as presented in chapter 3 and 4 of this thesis. This allows to analyse the density (power) of distinct frequency bins such as EEG slow-wave activity (SWA; 0.75-4.5 Hz) which represents a physiological reflection of homeostatic sleep regulation [39] (see also sections below).

1.1.2 Major brain structures and neurotransmitters involved in the sleep-wake cycle

The study of the brains of some of the victims from a pandemic influenza at the end of World War I, in particular of one form of the virus infection, the so-called Encephalitis lethargica, by Constantin von Economo, a psychiatrist and neurologist from Vienna, led to the recognition of the vital functions of the hypothalamus in the sleep-wake cycle [40]. What von Economo hypothesised more than 80 years ago about the sleep promoting functions of the anterior hypothalamus and the wake promoting functions of the posterior hypothalamus is still a central part of the current understanding of the functional neuroanatomy of sleep-wake regulation (Fig. 1.1.3).

As schematically depicted in figure 1.1.3, different nuclei in four major brain areas are involved in sleep-wake regulation and the projection of sleep and wake signals to the cortex. These areas comprise the brainstem, hypothalamus, thalamus and basal forebrain. As a superior regulatory structure in the sleep-wake cycle the suprachiasmatic nuclei (SCN) located in the anterior hypothalamus acts via neural or chemical circuits directly on other sleep-wake cycle relevant nuclei as mentioned thereafter and indirectly via the sympathetic neural system by regulating the nocturnal production and release or diurnal cessation of production and secretion of melatonin by the pineal gland. [16, 41, 42]

Neurons from the tuberomammillary neucleus (TMN) located in the hypothalamus and from the laterodorsal and pedunculopontine tegmental nuclei (LDT and PPT) as well as from the locus coeruleus (LC) and the dorsal raphe (DR) nucleus constitute the so-called ascending arousal system (AAS) which is responsible for the promotion of wakefulness via many neurochemical systems [43, 41, 44]. More recently, dopaminergic neurons of the ventral periaqueductal gray matter in the midbrain (not considered in figure 1.1.3) have been identified to be an additional neuronal part of the ascending arousal system [45]. The AAS has two different signal pathways to induce EEG desynchronisation and cortical arousal (wakefulness), respectively: a dorsal one through the thalamus and a ventral pathway through the hypothalamus and basal forebrain [42].

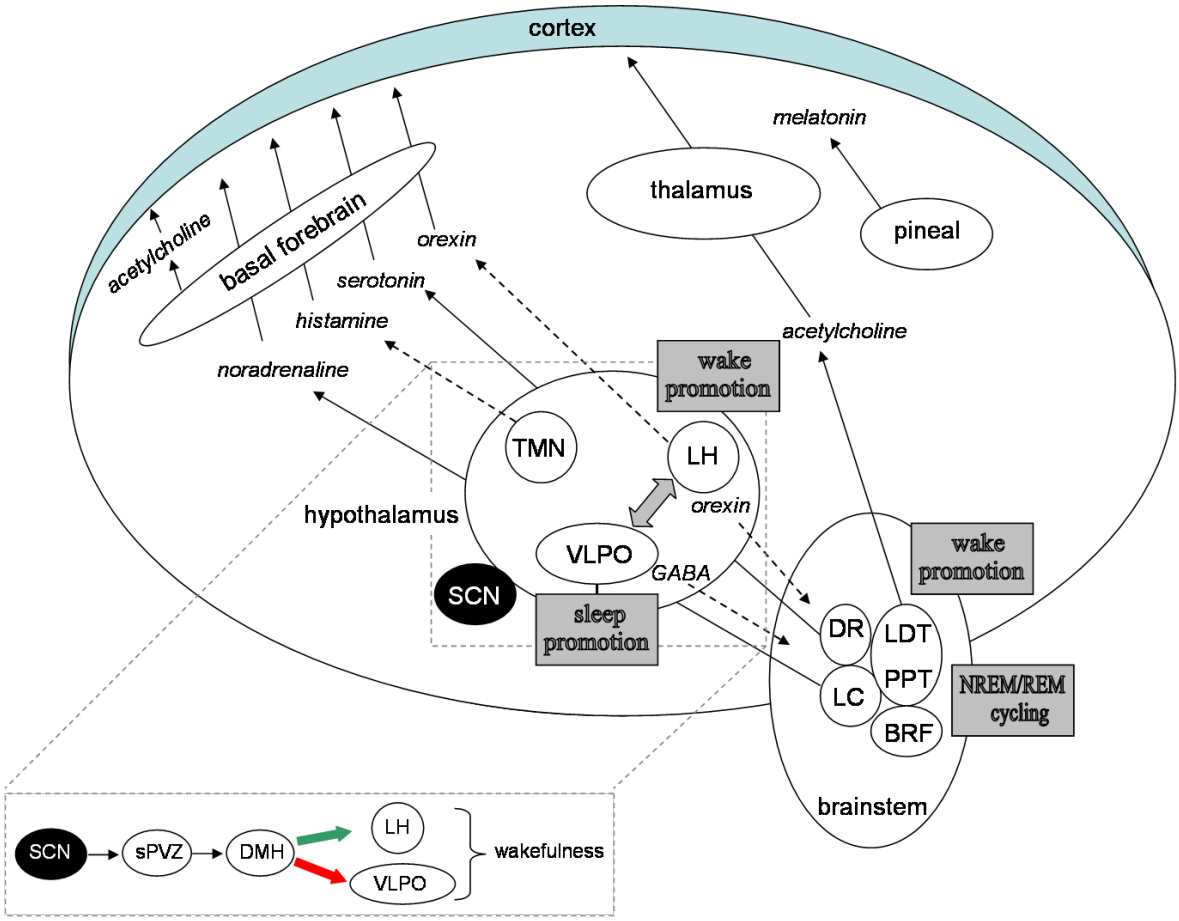

Figure 1.1.3: Schematic representation of the major interactions of sleep-wake cycle relevant brain structures, their interactions and involved neurotransmitters

Circadian regulation of the sleep-wake cycle originates in the suprachiasmatic nuclei (SCN) via direct chemical and neural projections to relevant wake and sleep promoting brain regions in the hypothalamus, basal forebrain, thalamus, and the brainstem. The panel at the bottom of the figure illustrates one recently suggested pathway according to which the SCN influences sleep and wakefulness by promoting wakefulness via the supraventriuclar zone (sPVZ) and the dorsomedial nucleus of the hypothalamus (DMH) leading to an activation of the wakefulness promoting structures (green arrow) and inhibition of the sleep promoting centres in the hypothalamus (red arrow) [46]. Additionally, the circadian production and release of melatonin by the pineal which is also controlled by the SCN likely modulates sleep-wake regulation. Supposedly, the ventrolateral preoptic nucleus (VLPO) and the lateral hypothalamus (LH) act as major sleep and wake promoting areas, respectively and the interaction between both leads to the segregation of sleep and wake states. Beside inhibition of the LH neurons the VLPO inhibits also the so-called ascending arousal system (AAS) being made of the tuberomammillary nucleus (TMN), dorsal raphe nucleus (DR), locus coeruleus (LC), and the laterodorsal and pedunculopontine tegmental nuclei (LDT and PPT) by the release of the neurotransmitter GABA (gamma-aminobutyric acid). Wakefulness is promoted by the neurotransmitter orexin (also known as hypocretin) synthesised and released by the lateral hypothalamus. Orexin inhibits sleep promotion by the VLPO and activates wake promotion by the basal forebrain and the AAS which in turn projects via different neurotransmitters to the basal forebrain and to thalamocortical areas. The ultradian rhythm of NREM-REM cycling during sleep is a consequence of the regulatory interaction of different nuclei in the brainstem, namely of the DR, LC, LDT, PPT, and brainstem reticular formation (BRF). (Figure adapted from Foster, 2005, p. 197)

Additionally to the ascending arousal system also neurons from the lateral hypothalamus (LH) are involved in wake promotion whereby neurons project to the AAS, the basal forebrain, and the ventrolateral preoptic nucleus (VLPO) through the release of orexin (=hypocretin). Cell lesions in the lateral hypothalamus cause narcolepsy, a very serious sleep disorder [47, 48, 7]. These well-coordinated arousal circuits are inhibited by the ventrolateral preoptic nucleus (VLPO) in the anterior hypothalamus. The VLPO promotes sleep by GABAergic fibres which inhibit the activity of the neurons of the AAS and the LH [49]. It has been shown that lesions of VLPO neurons leads to sleep fragmentation and insomnia [50] and it has been suggested that naturally occurring sleep fragmentation in healthy ageing is due to cell losses in the VLPO [28].

Some of the mentioned brain areas are not only involved in sleep-wake regulation but are also responsible for the cycling of NREM-REM sleep phases during a sleep episode [35]. Five brain nuclei in the brainstem, namely DR, LC, LDT, PPT, and BRF (brainstem reticular formation) provide the oscillatory switch approximately each 90 minutes between these two sleep phases.

1.1.3 Two-process model of sleep regulation

Humans exhibit a so-called monophasic sleep-wake pattern with the major sleep episode occurring during the night (i.e. the dark phase) and wakefulness during the light phase. However, the timing of the nightly sleep episode is still rather individual as well as its duration. The main factors regulating the timing, duration and intensity of sleep have been formulated 30 years ago within the two-process model of sleep regulation. According to this model, the timing, intensity and consolidation of sleep and wakefulness depend on a circadian aspect (process C) and a homeostatic aspect (process S) and on their interaction (Fig. 1.1.4) [51, 52, 53]. Process C is dependent on a circadian pacemaker and follows a 24 hours rhythm and process S as a measure of sleep pressure is directly dependent on the duration of prior wakefulness and dissipates during NREM sleep [39].

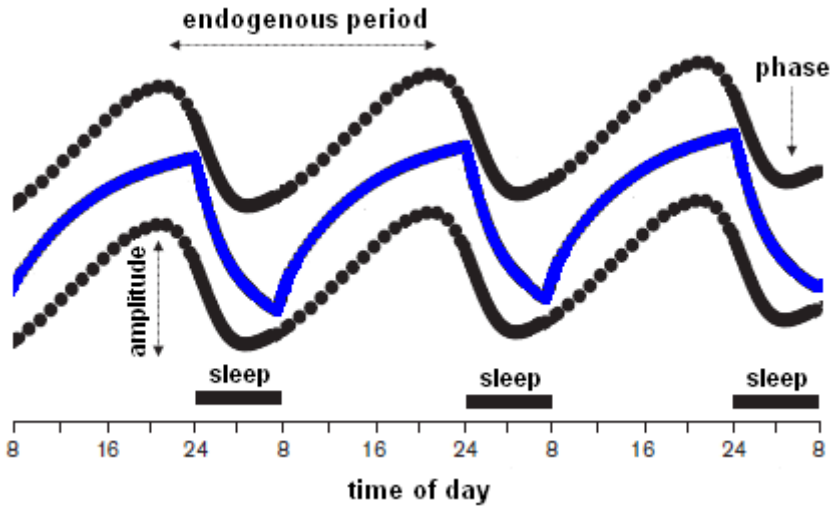

Figure 1.1.4: Scheme of the two-process model of sleep regulation

The homeostatic process S (depicted in blue) builds up exponentially during wakefulness. During sleep, process S dissipates exponentially. Process S is indicated by slow-waves during NREM sleep and measured by EEG slow-wave activity (SWA) in the delta frequency range of 0.75-4.5 Hz. The circadian process C (black sine waves) is independent of the history of prior wakefulness and follows - entrained by environmental signals called Zeitgebers - a 24 hour rhythm governed by the circadian pacemaker. These two processes act opponently: as sleep pressure (process S) is raising continuously during the day, the wake signal by process C is raising likewise in order to prevent sleep at an ecological inappropriate time during the circadian cycle [54, 55]. The oscillation of process S is limited by thresholds set by process C, i.e. sleep occurs when the wake promotion signal of process C weakens and wake up is preceded by circadian wake promotion.

Circadian process C The sleep-wake cycle is one of the most striking example of a biological rhythm. The circadian nature of this cycle is a result of a complex system of synchronisation of the physiology and behaviour of individuals with the environment. In humans such as in other mammals a master clock of the circadian system has been identified to be located in the SCN, a bilateral nucleus of approximately 20'000 cells located in the anterior hypothalamus [56, 57, 58]. The master clock ticks along an endogenous rhythm (so-called intrinsic period or freerunning period) generated by the SCN with a period length which is in general somewhat longer than 24 hours [59, 60]. Its rhythmicity is entrained to external time mainly by the environmental light-dark cycle. Thereby, different so-called Zeitgebers such as light as the strongest Zeitgeber but also behaviour entrain the endogenous circadian period generated by the SCN to the environment.

The effective timing of a circadian rhythm output (circadian phase) such as the sleep-wake cycle depends on many factors apart from the intrinsic period as shown schematically in figure 1.1.5. In particular, the sensitivity to light based on genetic differences and physical condition of the eye have modificatory effects on the sleep-wake behaviour and sleep timing respectively as apart from the image forming responses to light, the eye in mammals serves also encoding of the ambient level of light (irradiance) in order to mediate photoentrainment

of the SCN [43]. Thereby, not only image-forming photoreceptors (i.e. rod and cones) are responsible for irradiance perception but also recently discovered non-image forming retinal ganglion cells which express the photopigment melanopsin [61]. The contribution of either rods and cones or photosensitive ganglion cells to photoentrainment of the circadian clock may explain why some blind people still remain entrained to the light-dark cycle although their visual acuity is zero whereas bilaterally enucleated blind people suffer from the symptoms of a desynchronized biological clock such as sleep disturbances, reduced alertness and performance for example [62, 63, 64].

Apart from the health condition of the eyes, photoentrainment depends also from light intensity, light exposure duration, its spectral composition and its timing. For instance, it has been shown that a light pulse in the early morning has advancing effects on the circadian phase relative to external clock time and light applied in the evening results in a phase delay [65, 66, 67, 68, 69].

Photic information is subsequently transduced from the retina to the SCN through a monosynaptic pathway located within the optic nerve, the retinohypothalamic tract (RHT) [70]. Various chemical and neuronal output signals of the SCN synchronize behavioural rhythms (e.g. sleep-wake cycle, alertness, mood, etc.), endocrine rhythms (e.g. melatonin expression), and orchestrate balanced rhythmicity of peripheral oscillators located in different organs such as liver, kidney, and heart for example as illustrated in figure 1.1.5. Importantly, although the SCN is required for the coordination of the peripheral rhythmic output there is growing evidence that the peripheral circadian oscillations are self-maintained independent of the SCN and influenced by non-photic Zeitgebers such as feeding for metabolic activities in the liver for instance [71].

The timing and duration of human sleep as well as its structure (in particular REM sleep) depend on circadian phase which can be measured for instance by core body temperature or melatonin secretion [72] both of which are well studied hands of the circadian clock. Dim-light melatonin onset is known as a very reliable marker of circadian phase position indicating the raise of the biological night [73, 74, 75]. Melatonin is produced by the pineal gland and its secretion is attenuated by light: During the day, when the SCN is activated by light, melatonin secretion from the pinealocytes is inhibited via a multisynaptic pathway whereas during low activity phases of the SCN during the night, plasma melatonin levels increase leading to the typical nocturnal profile of melatonin, also known as «dark» hormone (and often falsely called sleep hormone) [76]. Healthy human sleep occurs when melatonin is rising and core body temperature is decreasing due to a diminution in heat production in combination with a melatonin mediated increase in blood flow and thus heat loss in distal extremities (i.e. thermoregulatory cascade) [77, 78]. Under entrained dark-light cycle conditions sleep starts approximately 6 hours before and ends 2 hours after the nadir of core body temperature [79]. Thereby, circadian phase dependent sleep propensity is lowest few hours before usual bedtime whereas it is at its maximum close to the nadir of the core body temperature [80]. At first glance this regulation seems paradoxical but it is in

accordance with the suggested opponent roles of the circadian and homeostatic processes in the regulation of sleep and wakefulness as afore mentioned (see Fig. 1.1.4) in order to avoid sleep or wake at inappropriate times of the day (i.e. early onset of sleep because of high homeostatic sleep pressure in the evening or early awakening in the morning due to dissipated homeostatic sleep drive during prior sleep episode) [54, 55].

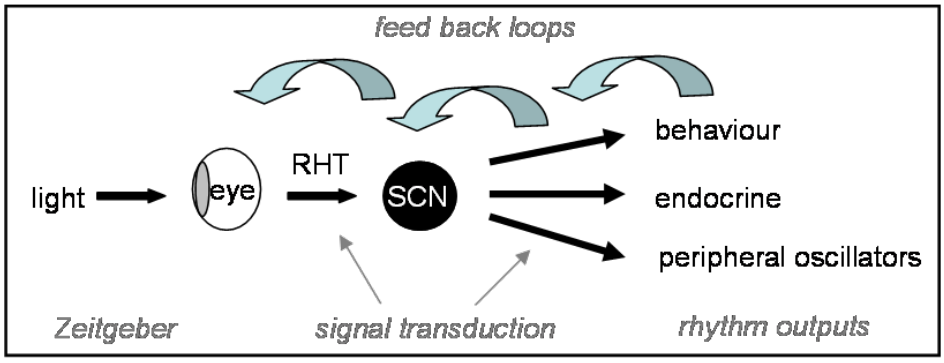

Figure 1.1.5: Schematic illustration of circadian signal entrainment and transduction (adapted from Foster 2005, p. 199)

The current view of the circadian system in mammals consists of a complex system of signal transduction and output pathways. Circadian rhythms are controlled by a pacemaker located in the suprachiasmatic nuclei (SCN) in the anterior hypothalamus. The intrinsic circadian period generated by the SCN is synchronised to the environment by so-called Zeitgebers out of which light is of major importance. After detection by the eyes the light signal is transduced to the SCN along the retinohypothalamic tract (RHT). The circadian timing information thereafter is transmitted by various pathways to respective effector systems leading to entrained behaviour such as sleep and wake states, synchronised endocrine output such as melatonin production and secretion, and rhythmic coordination of a network of peripheral oscillators located in both, central and peripheral tissues such as the liver for example. Finally, feedback from all output rhythm systems in particular to the SCN and from there backwards to input signal transduction sustain adequate coordination of the entire organism with its environment.

Homeostatic process S The delay of the sleep time and a prolongation of the previous wake time respectively, challenges a rebound in sleep intensity as measured by EEG slow-wave activity in particular in frontal brain regions [81, 82, 83, 84, 85]. This recuperative response to sleep deprivation has been attributed to the homeostatic aspect of sleep regulation (i.e. process S). In contrast to the circadian process C there is no neural tissue found so far for the control of process S. It has been suggested that adenosine plays a role as endogenous somnogen associated with homeostatic sleep regulation [86, 87]. This assumption is based on the observation that adenosine levels rise throughout the brain during prolonged wakefulness and decreases during sleep [88, 89]. Moreover, adenosine antagonists such as caffeine decrease sleepiness and increase alertness as well as EEG arousal, respectively [90]. However, there are many other potential sleep substances, which play an important role in homeostatic sleep regulation such as prostaglandin D2 etc. [91, 92].

It has been demonstrated that the time course and intensity of process S can be derived

from EEG recordings during NREM sleep. In particular, slow-wave sleep (SWS) and SWA respectively, has been shown to be a gaugeable indicator of process S [39, 85, 84]. During a night sleep episode, SWA declines continuously across sleep cycles until the accumulated sleep pressure during the previous wake episode is completely dissipated and sleep need is satiated. It is therefore important to note, that SWA is not only considered as an indicator for the dissipation of process S but also for the accumulated sleep need (sleep pressure) during wakefulness. According to the two-process model of sleep regulation (Fig. 1.1.4 above) process S increases during wakefulness until a threshold time point set by the circadian process C is reached and sleep occurs. During sleep, process S dissipates and wake up occurs due to another threshold time point set by the circadian process of sleep regulation [51, 53]. The decay and rise rate of process S can be calculated by fitting exponential functions to empirical SWA data. While the decay can be estimated by fitting an exponential decay function to the average SWA value of each NREM-REM sleep cycle (see also chapter 3 and 4) the rise rate can be calculated on the basis of a saturating function fitted to time points of SWA activity values of a baseline and a recovery night after several hours of sleep deprivation [93]. The two-process model established by Borbély in 1982 [53], experimentally confirmed by Dijk et al. in 1987 [39], was further evaluated quantitatively as well as refined by Achermann and colleagues in 1993 [84]. The most striking new aspect of the refined model beside others was that process S was allowed not only to increase during wakefulness but also during sleep (e.g. during REM sleep episodes, wake and movement periods, and even during NREM sleep).

Homeostatic sleep regulation is subject specific and varies across lifespan as indexed by large interindividual variations and profound age-related changes of the amount of SWS and the level of SWA. Across the human lifespan SWS and SWA is highest during prepuberty and decreases thereafter while sleep fragmentation through nocturnal awakenings increase. Additionally, it has been shown that also sleep propensity decreases with age independent of circadian phase. Thus, healthy ageing goes along with various changes in homeostatic sleep regulation as expressed by NREM sleep and SWA in particular [94, 95, 96, 97, 98, 99, 100, 101, 102].

In the context of the still unresolved research question «why do we sleep?» the synaptic homeostasis hypothesis has been elaborated recently. This hypothesis states, that sleep and in particular SWS serves to downscale synaptic strength acquired during wakefulness in order to achieve energetic sustainability and space efficiency in many brain areas to benefit memory processing and neuroplasticity [103, 104]. In this sense, hyperpolarization and synchronization of thalmocortical and cortical neurons apparent in high-amplitude slow-wave sleep [105, 106] appears to weaken as cortical synaptic strength decreases thus leading to the decreasing profile of slow-wave activity commonly observed during a usual night sleep episode [107].

The two-process model of sleep regulation has been proven to be of major importance in ex-

plaining diverse aspects of sleep physiology. For instance, sleep propensity (i.e. the latency to fall asleep) has been proven to be dependent on circadian phase while the intensity of NREM sleep seems to be rather independent of process C and is mainly modulated by the prior history of sleep and wakefulness [39, 85, 84]. Moreover, the model allows to hypothesise on possible reasons of sleep-wake cycle abnormalities such as in circadian rhythm disorders (e.g. jet-lag, advanced sleep phase syndrome etc.) and in many psychological disorders (e.g. major depression, schizophrenia etc.) and accounts also for natural occurring age-related changes in the sleep-wake cycles [108, 109, 110, 111]. In connection with the content of this thesis two concepts developed on the basis of the two-process model have to be shortly emphasized: first, the S-deficiency hypothesis and second, the model for the delayed sleep phase preference in adolescents. The S-deficiency hypothesis in depression was formulated by Borbély and Wirz-Justice model in connection with the frequently observed sleep disorders in major depression and remarkable therapeutic successes with sleep deprivation (see following section) [112, 113]. Borbély and Wirz-Justice thereby argued that sleep-wake abnormalities may be due to a damped increase of process S during wakefulness expressed by decreased SWS and SWA compared to healthy controls which could be boosted to normal level through sleep deprivation. The recently developed model for the delayed sleep phase preference during adolescence considers developmental changes in the circadian and homeostatic processes of sleep regulation [114]. According to this model, circadian phase is delayed and leads to a longer lasting wake up signal in evening and accordingly to a later wake up signal in the morning whereas at the same time resistance to sleep pressure increases. Both processes lead to the commonly observed late bedtimes in adolescents combined with long sleep durations on free days. In summary, there are various changes in sleep regulation which may result in sleep-wake cycle abnormalities such as altered homeostatic build-up or dissipation of sleep pressure, increased or decreased circadian amplitude, shifted circadian phase, and misaligned phase relationships between internal and external clocks (i.e. bed times not in line with endogenous melatonin phase etc.)(see also Fig. 1.2.2).

1.1.4 Natural changes of sleep patterns and chronotype

During a human lifespan sleep undergoes a number of specific changes. These changes encompass sleep duration, sleep architecture, electrophysiological features, and sleep timing. Age and gender as well as developmental stage have strong influences on sleep. In particular for women the infradian rhythm of the reproductive state has been reported to be an additional factor mediating sleep changes as marked influences of pregnancy, the phases of the menstrual cycle, menopause and associated hormonal changes on sleep quality has been observed for example [115]. Thus, as this thesis comprehends sleep in women special emphasis has been given to this factor as study participants were only admitted to the laboratory during their follicular phase (days 1-5 of the menstrual cycle; see chapter 3 and 4).

Sleep duration is highest in newborns with up to 19 hours per day decreasing thereafter progressively to about 10 hours at the age of 5 years [116]. In healthy adults the average sleep

time turns into 7.5 - 8.5 hours per night [33]. The occurrence of sleep bouts changes from polyphasic events in newborns and infants to the typical one night bout in early childhood and thereafter [116]. However, independent of age people exhibit their individual preference for the duration of their sleep and people exhibiting a sleep regulation in the extremes are referred as short and long sleepers in the current scientific literature [117]. The latter live on a longer biological night than short sleepers as measured by the nocturnal interval of plasma melatonin, core body temperature, and cortisol [118]. Hence, a contribution of the circadian pacemaker to the individual preference of sleep duration is very likely.

Sleep architecture in respect to NREM and REM sleep also changes throughout lifespan. Slow-wave sleep and SWA show a marked drop during adolescence and continue to decline thereafter as a function of age [102, 94, 38]. Moreover, independent of age, women tend to exhibit higher levels of slow-wave sleep and slow-wave activity than men [97]. Although morphological skull differences between women and men have been originally suggested leading to a divergent electrical conductivity [119], the causes of these gender differences remain largely unknown. The proportion of REM sleep decreases from around 50% at birth to 20-25% during adolescence and middle age.

Most striking changes in sleep timing occur during puberty and with aging. Thereby sleep phase preferences are typically delayed or advanced respectively resulting in a tendency to go to bed or wake up later or earlier [120]. People who tend to go to bed early and wake up early are commonly referred to as larks or early chronotypes, respectively. Owls in turn are people who usually go to bed late and - if social circumstances allow this - do get up quite late during the day. Owls are called late chronotypes and are more common than early chronotypes. However, the majority of people in western societies seem to be so-called intermediate chronotypes with a sleep-wake behaviour lacking extreme timings when compared to the socioeconomic demands of our modern society [121, 122].

Different questionnaires have been developed in order to assess chronotype. One of the first was the morningness-eveningness preference questionnaire (MEQ) and the diurnal type scale [10, 11]. Both questionnaire ask for sleep timing preferences in a qualitative manner without considering real life wake up and sleep times. A different approach in assessing chronotype was chosen by the recent development of the Munich Chronotype Questionnaire (MCTQ) where actual sleep timing during work days and free days are surveyed [122]. The scoring of the chronotype is represented by the midpoint of sleep during free days placing subjects on a continuum between early and late chronotypes in contrast to the discrete classification of the earlier questionnaires. Moreover, the MCTQ allows to gather insights into different aspects of real-life sleep-wake behaviour (i.e. sleep latency, sleep intertia, sleep debt acquired during working days etc.) whereas the other questionnaires allow to assess preferred sleep-wake behaviour. The chronotype survey as presented in chapter 2 of this thesis was based on the MCTQ.

Diverse investigations have so far elucidated factors which contribute to individual chronotype such as age, gender, instrinsic circadian period, and genetic differences [123, 124, 122,

125, 126, 120, 127, 128, 99]. Longer (> 24h) intrinsic circadian periods for example have been attributed to late chronotypes whereas early chronotypes have been shown to exhibit circadian periods shorter than 24 hours [60, 129]. Recent data also suggest that women have significantly shorter intrinsic circadian periods than men [130], which corresponds with later chronotype scores in men than in women [121]. The age-dependency of chronotype has been shown by various studies. Thereby, prepubertal children independent of sex tend to be rather early [131], during puberty sleep phase preference tends to shift to later clock hours and also an obvious difference between sleep duration on school days and weekends becomes obvious [132, 133]. Thereby, a correlation between pubertal development stage and later chronotype in females [134, 120] as well as longer sleep durations and later wake up times in pubertal girls compared to boys on free days have been shown [133]. Towards the end of adolescence, a noticeable anew sleep phase advance is observed as shown by Roenneberg and colleagues [121]. They suggested that the age- and sex-dependent switch from delaying to advancing chronotype may represent a biological marker for the end of adolescence. Later in life, around the age of 50, humans tend to further advance sleep phase preference towards earlier chronotypes [135].

Scientific literature provides much information about changes in chronotype and sleep patterns of children and young adults. However, the ages covered by these studies mainly lack transitionary years between adolescence and adulthood where the above mentioned switch from delaying to advancing chronotype occurs. Moreover, both, age and the evident changes of the psychosocial life of adolescents compared to prepubertal children and adults do not account sufficiently to this change and recent studies gave evidence for the involvement of physiological parameters in sleep timing shifts particularly during maturation and the transition to adulthood [136, 137]. However, it still remains unknown whether there is a relationship between puberty and this change in sleep timing preference. In chapter 2 of this thesis we therefore investigated the question whether the onset of menarche, as physiological pubertal marker, is associated to the changes in sleep phase preferences during female maturation and adulthood.

1.2 Major depression

1.2.1 Neurobiological aspects of major depression

According to the Word Health Organization (WHO) depression affects about 121 million people worldwide and holds the 2nd rank of diseases causing loss of productive life (DALYs) in the age category 15-59 years worldwide [138]. Depression has many facets: the illness can develop at any age and it can be manifested episodically over weeks or years or - as observed far more often - have a recurrent character [139, 140, 141]. Epidemiological studies have shown that the risk for depression is gender and age dependent [142, 143, 144]. Whereas in men the risk for depression increases during puberty and remains fairly constant throughout life, women bear an up to twice as higher risk than men to develop major depression between early adolescence until the mid-50s with greatest risk for depressive illness during postpartum or perimenopausal periods [145, 146]. As during childhood, after experiencing menopause the risk decreases to similar levels as for men in the same age categories. It has been suggested that this gender difference is due to the dramatic estrogen fluctuations during the female reproductive lifecycle which in turn may impact the levels of neurotransmitters believed to be involved in the pathophysiology of depression (i.e. monoamines such as serotonin, noradrenaline, and dopamine)[147, 148].

Despite its high prevalence and socioeconomic impact as well as the considerable research efforts during the past decades, the knowledge on the aetiology and pathophysiology of major depression remains rather fragmented [149, 150, 151]. According to epidemiological twin, and family studies, depression is a highly heritable disease bearing an inheritance risk of about 40% but to date no specific gene abnormalities have been identified presumably due to the complexity of the illness and the possible involvement and interaction of many genes [152, 151, 153, 154, 155, 156, 149]. Furthermore, vulnerability to depression is also reported to be caused by environmental factors such as stress, emotional trauma and viral infections and their interaction with genetic predisposition [157, 153, 158].

Major limits in highlighting the causes and effects of depression are the quite idiopathic occurrence of the illness, the technical difficulties in tracing complex pathological changes in the brain, the limited power of animal models in mimicing common symptoms of depression such as guilt, and the overlapping nature of depression symptoms with other psychiatric disorders. Yet, there is still a lack of a precise and biologically verifiable (objective) definition of major depression (MDD). Thus, the clinical diagnosis of major depression still depends on a syndrome-based, subjective-qualitative classification where the persistence for at least 14 days prior to clinical interview of either depressed mood or loss of pleasure and interest in association with at least four other symptoms such as inattention, fatigue, disturbance of psychomotor activity, sleep disturbances, change of appetite and weight, self-depreciation, and suicidal thoughts is required [159, 149]. As a result, MDD diagnosis includes a wide variety of heterogenic subgroups of patients. This situation is reflected in literature by

many contradictory results with reference to structural and functional neurobiological abnormalities and different findings for sleep characteristics in major depression indicating the existence of different endophenotypes in depression [160, 161].

However, based on post-mortem studies and neuroimaging techniques there is quite a broad agreement on the major brain areas most likely involved in mediating the symptoms of depression [162]. As schematically depicted in figure 1.2.1 these areas include the prefrontal cortex, hypothalamus, brainstem, and several areas from limbic system such as amygdala and hippocampus [149, 151, 163, 164, 165, 166]. Cortical areas and hippocampus may be responsible for more cognitive aspects of depression such as impaired memory processing, risk assessment, and feeling guilt whereas the amygdala and other related brain areas may be important in processing emotional stimuli and thus mediating fear, anxiety, and reduced motivation [151]. In depression, morphological changes in the depicted areas have been reported such as reduced grey matter volume in the prefrontal cortex and hippocampus [167]. The latter could be responsible for memory impairment as observed in depression.

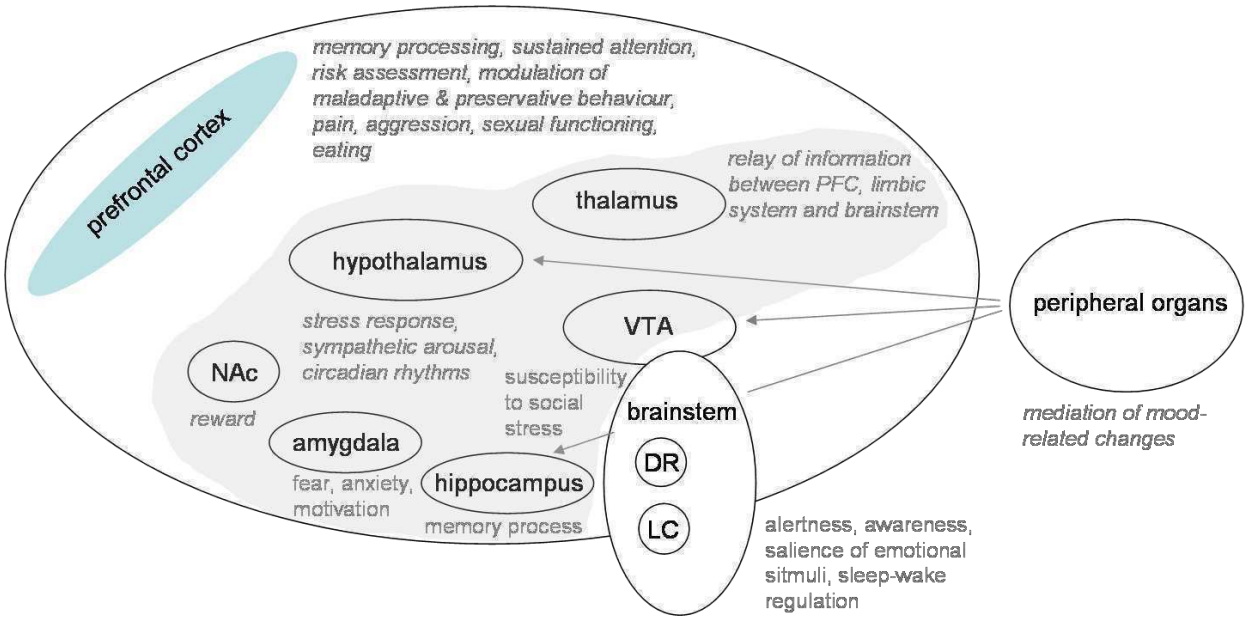

Figure 1.2.1: Simple schematic overview on brain areas involved in major depression

Beside structures in the frontal cortex also subcortical structures from the limbic system, brainstem areas and even hormonal information from peripheral organs such as from the adrenal glands (cortisol), stomach (ghrelin), and adipose tissue (leptin) are considered to shape the pathophysiology of major depression on the basis of complex circuitry systems. Some of the currently suggested main functions of the depicted brain areas are also mentioned (light grey) although such an attribution of functions is considered to be too simplistic vis-à-vis the emerged apprehension that major depression results from dysfunctions in a diffuse neural network [149]. Several of the mentioned brain regions are innervated by monoaminergic neurons (pathways not shown) such as by dopaminergic projections from the ventral tegmental area (VTA) and noradrenaline input from the locus coeruleus (LC) and serotonin secretion from the dorsal raphe (DR), both of the latter located in the brainstem. Brain regions belonging to the so-called limbic system are shaded in light grey. NAc = nucleus accumbens. (Illustration adapted from Krishnan et al. 2008)

Almost 50 years ago, the oldest hypothesis on the pathophysiology of depression has been formulated, namely the monoamine hypothesis [168, 169, 170]. According to this hypothesis, depression is caused by decreased monoamine levels in the brain. The monoamine hypothesis originated from the clinical observation that certain medications originally not dedicated to the treatment of depression elevated the mood of patients and subsequent insights that these medications elevate the levels of serotonin and noradrenaline. This discovery gave way to a revolutionized way of depression treatment and until these days the major part of antidepressant drugs are monoamine based (e.g. monoamine oxidase inhibitors). However, despite the wide response to antidepressants, the antidepressant effect is only observed with a long therapeutical delay and complete remission is only achieved in approximately 30-50% of all patients [151, 171]. Today, there is strong evidence that major depression is not caused by chemical imbalance but by disrupted neuronal information processing (i.e. the neurotrophic hypothesis on depression) and that antidepressants have an indirect effect on the recovery of mood by activating neural plasticity and connectivity through increased monoamine metabolism which in turn gradually improve neural information processing [172, 149, 166, 173].

In addition to the recent evidence on the involvement of changes in information processing networks the important contribution of chronic (social) stress to the pathophysiology of major depression has been recognised. Thus, stress has been demonstrated to decrease the expression of brain-derived neurotrophic factor (BDNF) in some limbic brain structures such as the hippocampus accounting for the atrophy of grey matter observed in these brain areas and thus leading to adverse changes in neural plasticity successively expressed in depressed mood and core endophenotypes of major depression such as cognitive impairment [174, 175, 167, 176, 177]. Moreover, increased activity of the hypothalamo-pituitary-adrenal (HPA) axis has not only been observed in patients with acute depressive states but also in recovered patients as well as in people at increased risk for the development of depression [178, 179, 180, 181]. It has therefore been suggested that elevated salivary cortisol levels, as a physiological measurement of the HPA axis' activity, may serve as predictor of future episodes of depression [182].

As mentioned above, many of the current knowledge points towards impairment of structural (and functional) plasticity and stress resilience as has been postulated in classical degenerative neurological disorders such as Parkinson's and Alzheimer's disease [183, 175, 176, 149]. Clinical observations and polysomnographic recordings show that major depression often goes along with sleep disturbances although sleep disturbances are neither depression specific nor a compulsory syndrome for the clinical diagnosis of the illness [159]. However, it has been estimated that more than 80% of patients with major depression suffer from insomnia and 15-35% show signs of hypersomnia [184, 185]. Yet, as a result of the current understanding of the importance of the hypothalamus and the circadian system in the sleep-wake regulation their roles as well as of related processes modulating the sleep-wake cycle (process C and process S, see above) have been suggested to be involved in the pathogenesis of major depression [186].

1.2.2 Current findings on sleep abnormalities in depression and related circadian and homeostatic rhythms

Over 80% of persons suffering from major depression report sleep abnormalities [187]. It has been shown that poor sleep quality and the development of major disorder have an inverse correlation as such that depression often leads to sleep disturbances and that primary insomnia is an independent risk factor for depression [188, 189, 190]. Subjective sleep disruptions include early awakening, prolonged sleep latencies, and not relaxing sleep for example. Extensive literature on objectively measured sleep parameters by electroencephalography confirm abnormalities in sleep architecture in depression including prolonged sleep latency, shortened rapid-eye movement sleep latency and increased REM sleep and decreased slow-wave sleep (as measured by SWA particularly in frontal brain areas) at the beginning of the night, and a higher rate of wake up episodes especially towards the end of the night [191, 185, 192, 193, 187, 194]. Interestingly, many of these observed alterations in sleep patterns in depression, in particular concerning slow-wave and REM sleep as well as increased sleep fragmentation are also present in normal healthy ageing which lead to the note that depression is alike precocious ageing with respect to sleep architecture [195]. A refinement of this consideration has been suggested as such that depression might bear sleep-related similarities to premature ageing only with restricted sleep patterns not encompassing slow-wave sleep and REM sleep density but sleep efficiency, total sleep time, intermittent time awake during sleep, and REM sleep latency [196].

Many studies show that sleep architecture is gender and age dependent in healthy as mentioned above [100, 197, 119, 97, 127, 198] and similar results have been found in connection with sleep disturbances in depression. Thereby, observed changes with reference to SWA include lower values in depressed patients compared to age and gender matched healthy controls until the age of approximately 50 years, thereafter no significant differences have been observed [199, 200, 201]. Moreover, the lowering of SWA in depression was mainly attributed to the first NREM-REM cycle leading to a lower delta sleep ratio (proportion of SWA of the first NREM-REM sleep cycle and the second) in depressed patients than in healthy controls which was suggested to represent a biological predictor of the recurrence of depression [202]. Additionally, lower SWA has been observed in men with depression compared to age matched depressed women [203]. However, apart from gender differences there is no general consent on observed changes in sleep architecture and sleep EEG (e.g. slow-wave activity) in depression compared to healthy sleep as various studies failed to demonstrate or confirm such changes [204, 205, 206, 207]. Nevertheless, the search for distinct EEG-variables that characterize the apparent variety of endophenotypes in depression is in progress as they may serve as biomarkers for the identification and progress of the illness and for the prediction of the response to antidepressant treatments [208].

In virtue of the prevalence of sleep disturbances in depression it is not surprising that several hypotheses on the underlying causes were developed. For instance, the early onset

of REM sleep in major depression has been attributed to an imbalance of pontine cholinergic/aminergic or an increased sensitivity to cholinergic neurotransmission, respectively [209, 210]. Furthermore, several clinical features imply disturbances of the circadian pacemaker and of processes regulating the sleep-wake cycle such as the diurnal and seasonal change of mood and the acute though short-term improvement of mood after sleep deprivation which is commonly used in practice in combination with medication in the therapy of major depression [189, 211, 212, 109, 213, 214]. This assumption is underlined by the observation that circadian misalignment as observed in jet lag or shift work for example may cause neuropsychiatric symptoms commonly also observed in depression such as reduced attention, impaired alertness, lack of energy, negative mood, and sleep disturbances. Hence, different related hypotheses were suggested accounting for changes either in the circadian or the homeostatic (sleep-dependent) mechanism as proposed by the two-process model of sleep regulation (Fig. 1.1.4).

Proposed changes as illustrated in figure 1.2.2 concerning the circadian pacemaker include phase or amplitude alterations such altered phase relationships of circadian rhythms (e.g. advanced endogenous circadian phase of cortisol nadir relative to sleep schedule), or reduced melatonin amplitude, or phase angle abnormalities between cortisol acrophase and dim-light melatonin onset for example [209, 215, 216, 217, 218, 219, 220, 221].

In contrast to circadian system alterations, the so-called S-deficiency hypothesis refers to a possible disturbance of homeostatic sleep regulation in depression [112, 113, 222]. Therefore, the impaired homeostatic increase of sleep pressure (process S) during wakefulness leads to an alleviated need for dissipation of process S as measured by lower SWA during sleep compared to healthy controls. This decrease of SWA leads successively to early REM onset due to reciprocal interactions between NREM and REM sleep activity.

Despite ample support for each of the mentioned hypothesis by clinical studies as mentioned above, the cause-effect relationship of abnormalities in the sleep-wake regulatory mechanisms (circadian pacemaker, sleep homeostat) and sleep disturbances observed in depression and depression per se remains unclear.

A major problem thereby are methodological issues since only a handful studies so far have attempted to investigate circadian rhythms such as the sleep-wake cycle in depression under unmasking conditions, i.e. conditions controlled for influences of Zeitgebers such as light, behaviour, posture, temperature etc. (see chapter 1.3) in order to measure endogenous vs. entrained expression of circadian rhythms. Most of these studies thereby considered patients with seasonal affective disorder (SAD) [223]. This apparent lack of chronobiological studies in depression may be mainly due to the enormous efforts needed in terms of recruitment of patients, time spent in the laboratory, and of monitoring devices. Furthermore, the participation in such studies is exhaustive thus implicating ethical considerations when studying patients with major depression. However, carefully designed studies in order to analyse and dissect circadian and homeostatic influences on the sleep-wake cycle in depres-

sion as presented in this thesis (chapter 3 and 4) will certainly help to highlight the current understanding of possible disruptions in these processes in this prevalent mental illness.

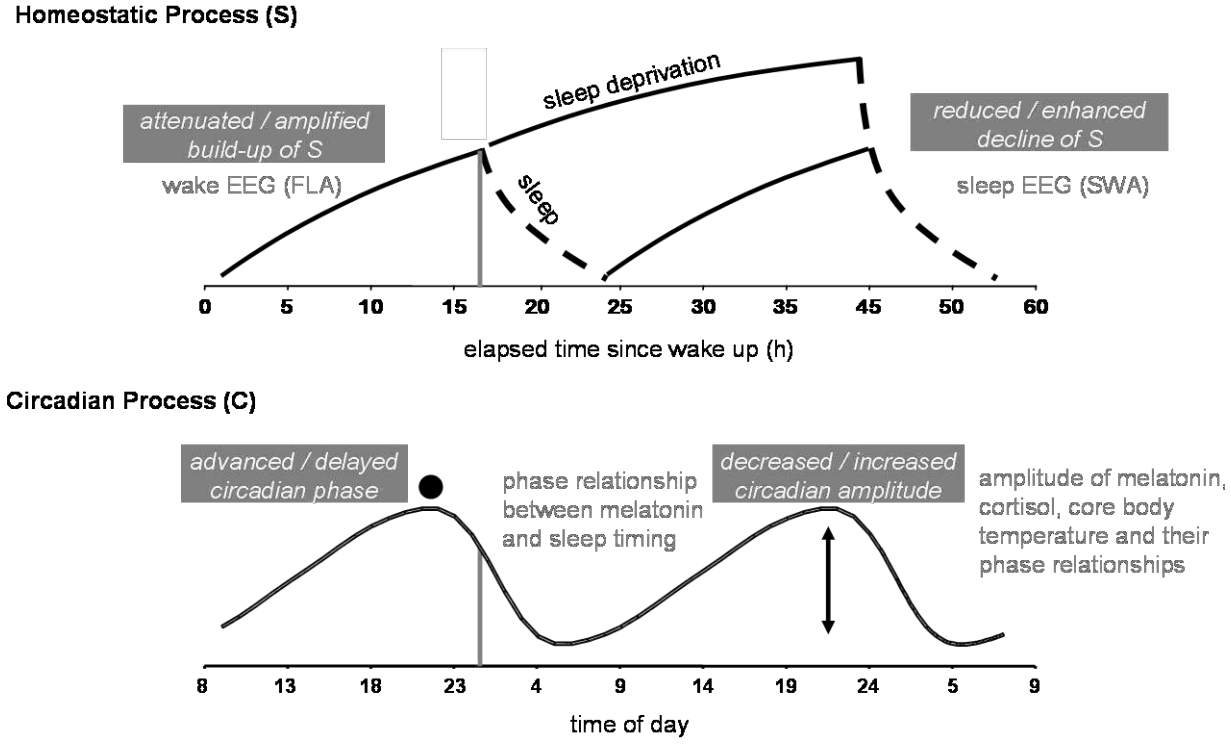

Figure 1.2.2: Possible alterations of the two-process model of sleep regulation in depression

The figure displays the homeostatic component (process S) and the circadian process C of the two-process model of sleep regulation. Possible changes which may occur in depression are indicated in gray shaded text boxes for each process. Furthermore, possible biological markers to get evidence of such disturbances are mentioned (grey coloured font). FLA = frontal low-frequency activity, SWA = slow-wave activity.

1.3 Methods to study circadian and homeostatic aspects of the sleep-wake cycle

Circadian and homeostatic aspects of sleep-wake regulation are highly interdependent. Endogenous circadian phase and amplitude is masked by several factors such as light, food, physical activity, and social stimuli. Hence, exact and detailed assessment of the influences of these processes and endogenous circadian rhythms require distinct experimental approaches. Over the last decades different protocols have been developed to study circadian and homeostatic regulation in human sleep, namely the forced desynchrony, the constant routine, and the nap or short sleep-wake cycle protocols [224, 55, 225, 226]. Among these protocols, the forced desynchrony is probably the most complicated and time consuming experiment where subjects live under artificially very long (28 hours) or short days (20 hours) for several weeks. The circadian system is thereby not able to entrain to these long (or short) days which results in the occurrence of sleep and wakefulness at different circadian phases during the entire 24-h cycle (i.e. desynchronisation of the sleep-wake rhythm from the circadian

pacemaker). Importantly, although sleep occurs ad libitum prior time awake until sleep onset remains mainly constant while circadian phase changes allowing thus the subsequent analytical segregation of the homeostatic and circadian contribution to a given variable of the sleep-wake cycle such as melatonin and slow-wave activity for example.

The aim of constant routine protocols is the stringent control of the environmental (i.e. light, temperature, etc.) and behavioral (posture, caloric food intake, movement, etc.) conditions in order to unmask endogenous circadian phase. Study participants thereby remain usually awake for 24 up to 60 hours in dim-light while staying in bed in a semi-recumbent position. In the time course of this sleep deprivation homeostatic sleep drive is progressively increasing while at the same time endogenous circadian phase is changing. This setting allows to analyse homeostatic sleep regulation after challenging process S through prolonged wakefulness and to assess unmasked circadian rhythms.

Despite these advanced experimental approaches to measure the relative contribution of circadian and homeostatic influences some variables of the sleep-wake cycle such as subjective mood, sleepiness, performance and alertness for example still exhibit a varying degree of interaction between circadian and homeostatic influences [227, 228]. The application of a short sleep-wake cycle protocol (nap protocol) with 2.5 hours awake and 1.25 hours of sleep over 24 hours or more helps to elucidate the impact of the circadian timing system on any variable of interest as homeostatic sleep pressure is kept at rather low levels.

The laboratory part of this thesis (chapter 3 and 4) comprised a sleep deprivation condition and a short sleep-wake cycle (nap) setting both under constant routine conditions as shown in figure 1.3.1. Study participants remained awake for 40 h or followed a short day protocol in which they were scheduled to be awake for 2.5 h and asleep for 1.25 h for 40 hours between a 8-h baseline and a 8-h recovery night of sleep.

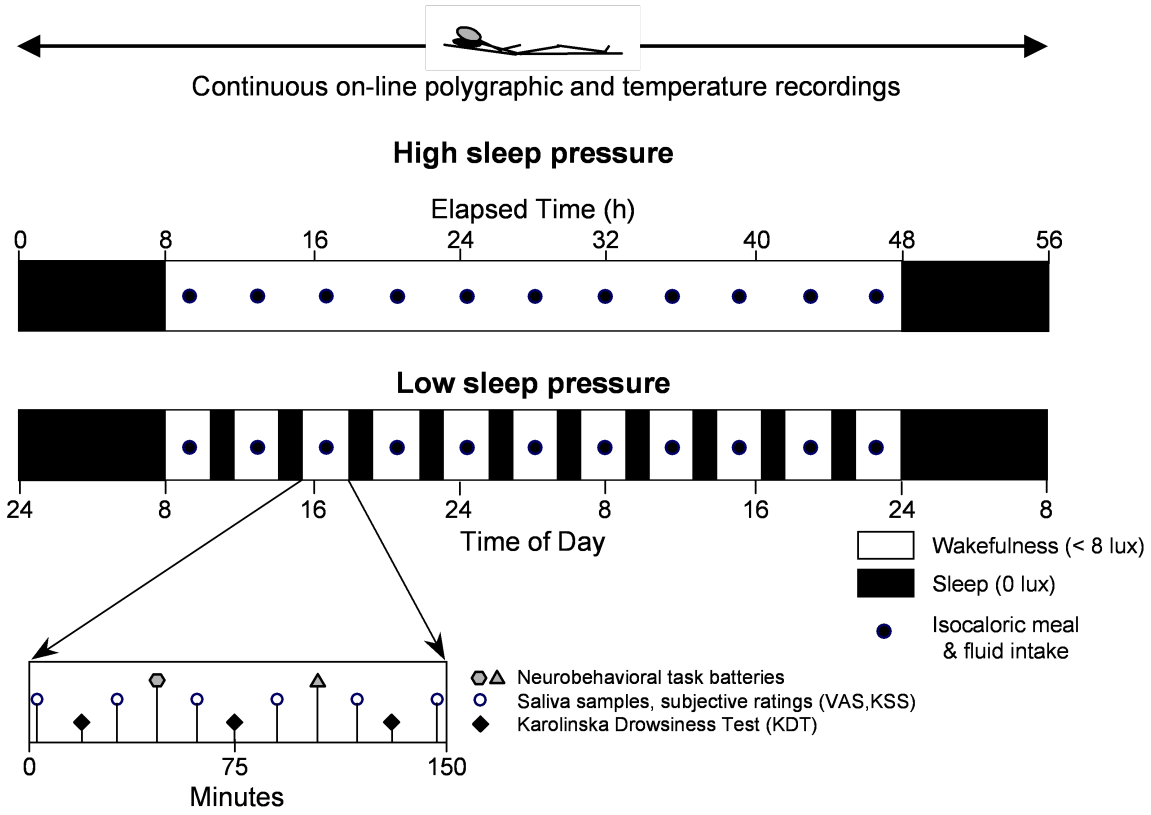

Figure 1.3.1: Study protocol designs

The figure illustrates the two sleep-wake cycle manipulating conditions applied in the laboratory part (chapter 3 and 4) of this thesis. The high sleep pressure protocol challenges the sleep homeostatic part of sleep regulation while the low sleep pressure protocol imposes a short day regime allowing for sleep episodes at different circadian phases. Activity levels are kept minimal since study participants stay in bed during the entire duration of both study protocols (56 hours) though in a semi-recumbent position during wake episodes. Food and fluid are provided to study participants at evenly spaced intervals; light is kept constantly under 8 lux while awake and is turned off during sleep episodes. During wake episodes different variables are measured in evenly spaced intervals such as salivary melatonin and subjective sleepiness.

1.4 Objectives and structure of the thesis

The general objective of this thesis was to elucidate sleep-wake regulation in women with respect to sleep phase preference during maturation and major depression. In particular, the first part, chapter 2, aimed to further expand the knowledge about the age dependency of chronotype and in particular, the association between female pubertal physiology and the observed delay of the sleep-wake cycle during the transition from adolescence to adulthood as expressed by shifting sleep phase preferences. The following working hypothesis was thereby tested:

1. Changes of sleep phase preferences during late adolescence are not only related to

chronological age and associated changes of psychosocial demands but also dependent on physiological maturation.

The study was based on a chronotype survey among 1'187 females aged 5-51 years. Habitual sleep (and wake) timing during work (school) days and free days, date of birth, and the date of the onset of menarche, considered as pubertal marker, were retrieved. The distance measured in years from the actual age to menarche was thereby taken as a physiological variable that may affect the change in sleep phase preference.

The aim of the second part of this thesis was the assessment and quantification of homeostatic and circadian aspects of sleep-wake regulation in young women with major depression as compared to young and older healthy women. Homeostatic sleep regulation was analysed by means of EEG slow-wave sleep and slow-wave activity from 12 electrodes along the anterio-posterio axis. Circadian aspects of the sleep-wake regulation were additionally assessed by salivary melatonin and subjective sleepiness ratings. In particular, the following working hypotheses were tested:

1. Depressive volunteers show a significant reduction in circadian amplitude, as indexed by the circadian rhythm in salivary melatonin, compared to age-matched healthy controls.

2. The circadian wake-promoting signal in the early evening is significantly reduced in young depressed women, as indexed by more sleep during nap episodes scheduled during this time of day, compared to healthy young but not compared to healthy older women.

3. Depressive patients show a significant different phase position (either advanced or delayed) of the circadian rhythm in salivary melatonin compared to both healthy control groups.

4. Similar to healthy older women, young female depressive show a significant reduced response to sleep deprivation in frontal brain regions, as indexed by slow-wave activity during NREM sleep, compared to healthy young women.

Two protocols as depicted in figure 1.3.1 were applied to healthy volunteers in a cross-over design. In contrast, due to the exhaustive nature of these protocols the majority of the women with major depression participated only in one protocol which was randomly assigned. Both protocols started with a 8-h baseline night and ended with a recovery night either after a 40-h sleep deprivation or a multiple nap setting over 40 hours. On one hand, sleep deprivation challenged homeostatic sleep pressure which allowed comparing homeostatic sleep regulation characteristics in the three groups. The nap protocol on the other hand served to assess the influence of circadian phase on sleep without masking homeostatic influences. Both protocols were conducted under constant routine conditions (see above).

2 Consequences of the timing of menarche on female adolescent sleep phase preference

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Abstract

Most parents experience their children's puberty as a dramatic change in family life. This is not surprising considering the dynamics of physical and psychosocial maturation which occur during adolescence. A reasonable question, particularly from the parents' perspective, is: when does this vibrant episode end and adulthood finally start? The aim of the present study was to assess the relationship between puberty and the changes in sleep phase preferences during female maturation and adulthood by a cross-sectional survey. The results from 1'187 females aged 5 to 51 years based on self-report measures of sleep preferences on weekdays and on free days as well as the occurrence of menarche, show that in contrast to prepubertal children, adolescent females exhibit a striking progression in delaying their sleep phase preference until 5 years after menarche. Thereafter, the sleep phase preference switches to advancing. The current study provides evidence that a clear shift in sleep-wake cycles temporally linked to menarche heralds the beginning of adult-like sleep-wake behaviour in women and can be used as a (chrono)biological marker for the onset of adulthood.

2.1 Introduction

Most parents experience their children's puberty as a dramatic change in family life. This is not surprising, considering the dynamics of physical and psychosocial maturation, which occur during adolescence. A reasonable question, also from the parents' perspective, is: when does this vibrant episode end and adulthood finally start?
Human sleep-wake behaviour undergoes a 24-h rhythm, which is governed by the biological clock located in the suprachiasmatic nuclei[59]. However, individual sleep and wake time preferences are fairly diverse due to genetic, environmental and age-related factors, resulting in different individual timing (phase position) for early chronotypes (larks) and late chronotypes (owls)[229, 99, 230, 129, 231, 232, 233].
One striking characteristic of adolescence is a marked delay of sleep phase preference and an enhanced sleep duration on weekends that contrasts with sleep timing during weekdays [126, 234, 133]. As wake times during weekdays are fairly constant because of school times, and only bedtimes shift later, a considerable sleep deficit accumulates prior to the weekend. This deficit has to be caught up over the weekend, and is attempted by a temporal shift of wake and sleep times. The difference in sleep timing preference between weekdays and weekends has been aptly described as social jetlag [235].
Apart from the more obvious psychosocial factors, delayed phase preference in adolescents may actually be related to changes in the phase of the circadian timing system. A later pubertal development stage coincides with a later chronotype, particularly in females[120, 134]. In contrast to the end of the biological maturation (puberty), which is associated with a stop in physical growth, the end of other maturation domains of adolescence such as developing cognitive skills (e.g. formal operational thoughts) and psychosocial competence (e.g. identity formation) is yet not well defined. From this perspective, the beginning of adulthood still remains a fuzzy area. Recently, Roenneberg et al.[121] have suggested that the age- and sex-dependent switch from delaying to advancing chronotype may represent a biological marker for the end of adolescence.
However, chronological age alone does not give an accurate explanation for the physical and behavioural dynamics that occur before the transition from adolescence to adulthood. Thus, it remains unclear whether there is a relation between puberty and this switch in sleep timing preference at the end of adolescence. Here we aimed at assessing a relationship between puberty and the changes in sleep phase preferences during female maturation and adulthood.

2.2 Methods

Our study is based on a survey among 1'187 females aged 5 to 51 years (mean 23.7 years, SD= 9.5 years; median= 20 years; see supporting Fig. S1) of whom 2% were nursery girls, 40.5% high school students, 16.2% university students, and 41.3% of unknown occupation.

44.7% of the study participants were surveyed during the summer months (June–August), 33.6% during winter (December –February), 11.9% during spring (March–May), and 9.8% during fall (September–November).

Study participants were mainly recruited at high schools and at the University of Basle, Switzerland. Schoolmasters and teachers as well as University tutors were asked for approval and support of the survey. In addition to the survey on paper used at high schools and at the University an electronic version of the questionnaire was freely available online on our website (www.chronobiology.ch). Approximately 80% of the questionnaires were distributed personally by our researchers, teachers, and University tutors whereas about 20% were filled out anonymously online. Apart from the latter, study participation was either with verbal or written consent from the parents (nursery children). The study procedure and questionnaire were approved by the local Ethics Committee of Basle (EKBB), Switzerland, and all procedures conformed to the Declaration of Helsinki.

Chronotypes were assessed by the Munich Chronotype Questionnaire (MCTQ) [122]. The chronotype score MSF_sc was used to analyse differences in sleep phase preferences (Fig. 2.3.1 and Fig. 2.3.2). MSF_sc is calculated by an adjustment of the midpoint of sleep on free days by the individual average sleep need throughout the week as described in the supplemental data to [121]. Furthermore, habitual sleep timing on weekdays and free days were investigated (Fig. 2.3.3). Along with the date of birth, the year and month of menarche occurrence was also requested. Menarche was chosen as a pubertal marker since it is a milestone which remains mentally present throughout a woman's life. The distance measured in years from the present age to menarche was taken as a biological variable that may affect the change in sleep timing preference. Being age-dependent, this variable may serve as an indirect measure of the stage of pubertal maturation.

Although menarche timing was assigned by retrospective selfreported data, the observed mean age at menarche in our study of 12.96 years ± 1.41 years (median = 13 years; n= 1140) matched longitudinally measured age of menarche in Western societies ranging from 12.6 to 13.4 years rather well [236, 237, 238, 239]. Importantly, several studies have reported a moderate to high correlation between the real age at menarche and the menarche age recalled during adulthood and adolescence [240, 241, 242, 243, 244]. In order to avoid bias with reference to the exact month of occurrence of menarche, only the year of menarche was considered in the present analysis.

To account for interferences of individual sleep preference with social demands the difference between the midpoints of sleep on free days and on weekdays was calculated (Fig. 2.3.4). The assessment of mid-sleep time was calculated on the basis of indicated sleep onset and wake up times in the questionnaire. Average sleep duration (Fig. 2.3.5) was calculated by the formula (5 * sleep duration on weekdays + 2 * sleep duration during free days) / 7) according to the supplemental data to [121]. Sleep debt accumulated during the weekdays which is compensated on free days is calculated according to the supplemental data to [121] and illustrated with reference to distance to menarche (Fig. 2.3.6).

Distance to menarche as shown in figures 2.3.2-2.3.6 was calculated as the difference between actual age and age at menarche. 0 corresponds to survey participants who did not yet experience menarche but are considered to be close to it because of their actual age ranging from 10–17 years (mean = 12.83 years, SD = 1.53 years; median = 13 years; n = 23). The difference between the median age of this group and the median age at menarche in our sample is 0 and therefore the distance to menarche within the graph was considered accordingly. A distance to menarche of -7 years corresponds to prepubertal girls (mean age = 5.7 years, SD = 0.49 years; median = 6 years; n = 24). The distribution of the sample into distance to menarche classes is displayed in supporting figure S2.

The variables midpoint of sleep difference and MSF_sc were log-transformed in order to achieve a normal distribution of the data set (normal distribution confirmation by Kolmogorov-Smirnov test). Log-transformed values of these two variables were afterwards subjected to a one-way ANOVA each with the factor distance to menarche. Post-hoc comparisons were based on the LSMEANS procedure in SAS with a Bonferroni alpha level correction. Statistical analysis on the variables concerning bedtimes and wake up times, sleep duration as well as sleep debt were based on the Kruskal Wallis test. Posthoc comparisons were based on the Mann-Whitney U Test with a Bonferroni corrected alpha level. Statistical analyses were performed with the statistical packages SAS (version 9.1) and Statistica (version 6.1).

2.3 Results

Figure 2.3.1 displays the distribution of chronotypes in our sample. About 36.4% of the survey participants were late chronotypes whereas early and intermediate chronotypes accounted for 32.3% and 31.3%, respectively.

An examination of chronotype distribution with respect to pubertal maturation (i.e. distance to menarche) confirmed previous findings that evening types are more prevalent during adolescence and morning types in children as well as in females above 30 years (supporting Fig. S3).

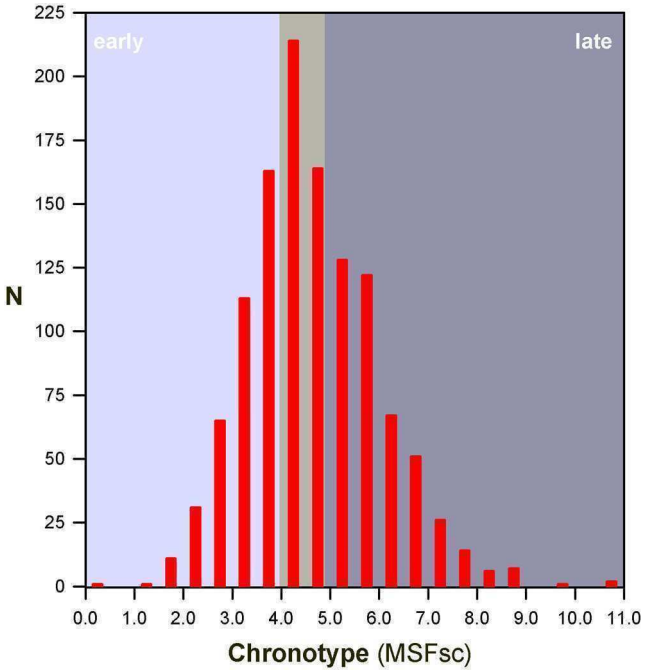

Figure 2.3.1: Chronotype distribution within the study sample

The MSF_sc score reflects the midpoint of sleep and therefore time of day (N = 1187).

As expected, prepubertal children exhibited a relatively early sleep phase preference compared to females during developmental maturation (Fig. 2.3.2). A peak value in sleep phase preference occurred 5 years after menarche and was therefore chosen in the following as reference point for post-hoc analyses unless mentioned otherwise. After this time point chronotype advanced progressively with years since menarche. One-way ANOVA yielded significance for the factor distance to menarche ($p < 0.0001$). Post-hoc comparison tests yielded that MSF_sc at -7 years to menarche ($p < 0.0001$), close to menarche ($p < 0.05$), 1 year after menarche ($p < 0.001$), 21.5 years, and more after menarche ($p < 0.0001$) were significantly different from the value at 5 years after menarche. Additionally, the difference of the MSF_sc at 16.5 years after menarche to the peak at 5 years after menarche was found to be close to significance with $p = 0.0505$.

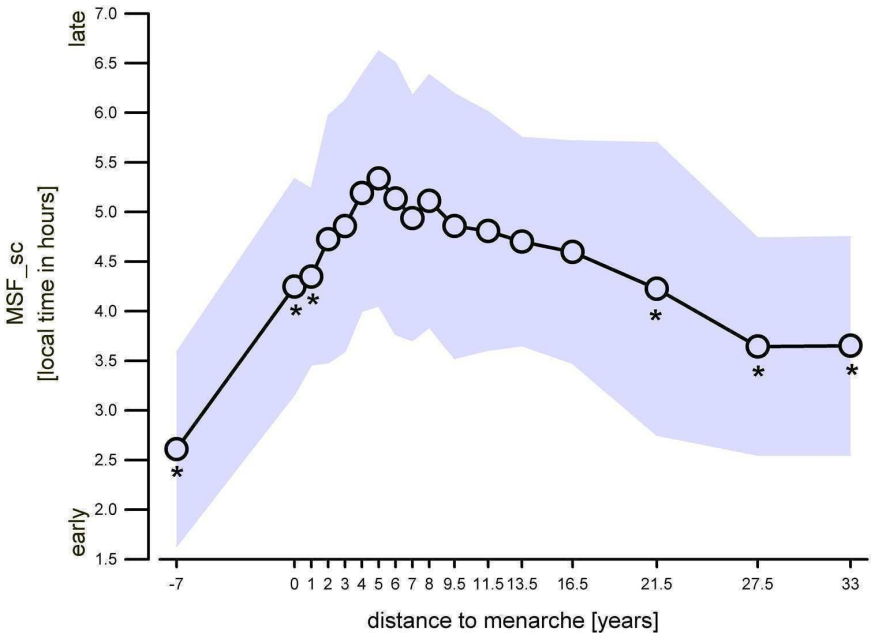

Figure 2.3.2: Development of sleep phase preference with reference to pubertal maturation

*Chronotype as indexed by the MSF_sc value is a measure for sleep phase preference. MSF_sc represents local time in hours. Shaded area represents ± SD; * indicate significant values compared to 5 years after menarche (Bonferroni adjusted alpha levels, for further statistics please see text).*

Habitual sleep and wake up timing on free days and weekdays are shown in figures 2.3.3a and 2.3.3b. Kruskal Wallis test yielded significance for each of the variables for the factor distance to menarche ($p < 0.001$). Post-hoc comparisons of bedtimes during the weekdays showed no significant difference between 2 years and 33 years after menarche. Taking the reference point (5 years after menarche) into account, only the bedtimes before the occurrence of menarche and 1 year after menarche differed significantly ($p < 0.003$). In contrast, wake up times during weekdays 5 years after menarche differed significantly from several wake up times at earlier and later maturation periods (-7, 9.5, 11.5, 13.5, and 16.5 years after menarche; $p < 0.003$). Bedtimes during free days revealed significant differences of the distance to menarche classes at -7, 0, 1, 2, 13.5, 16.5, 21.5, 27.5, and 33 years compared to 5 years after menarche ($p < 0.003$). Regarding the wake up-times during free days there were significant differences at -7 years to menarche and from 9.5 to 33 years after menarche compared to 5 years after menarche ($p < 0.003$).

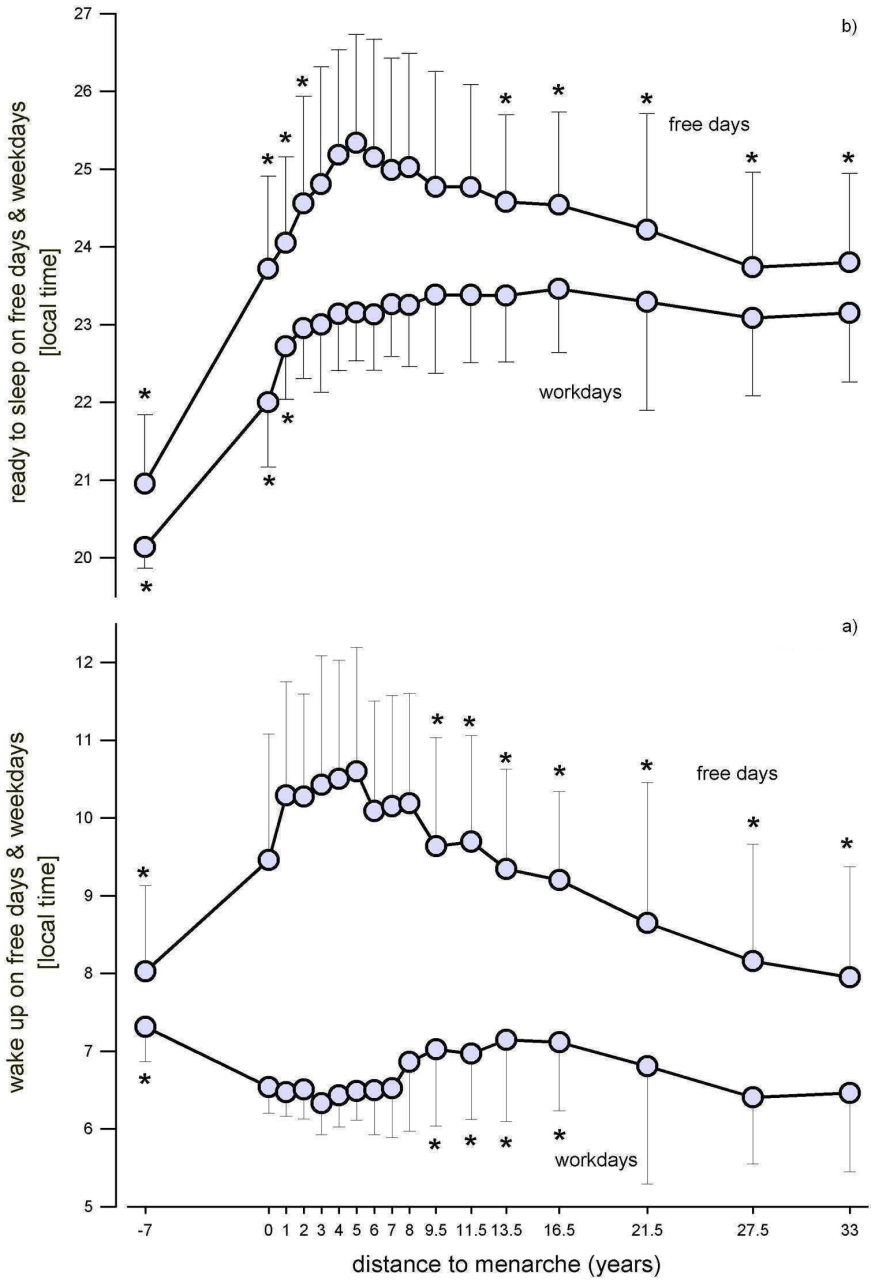

Figure 2.3.3: Sleep and wake up times during free days and weekdays with reference to pubertal maturation

*Mean values; N = 1187 ± SD; * indicate significant values compared to 5 years after menarche (Bonferroni adjusted alpha levels, for further statistics please see text).*

Prepubertal children exhibited a small shift in sleep timing preference between free days and weekdays (Fig. 2.3.4). As shown in Fig. 2.3.2, a delayed sleep phase preference was observed after the occurrence of menarche, which progressively increased until 5 years after menarche where the mid-sleep difference between weekdays and free days reached a maximum of about 3 hours on average (Fig. 2.3.4). Afterwards, a progressive decrease of the midsleep difference was observed. One-way ANOVA yielded significance for the factor distance to menarche ($p < 0.0001$). Post-hoc comparison showed that mid-sleep difference at -7 years to menarche and from 9.5 years and more after menarche were significantly shorter as the peak in mid-sleep difference at 5 years after menarche ($p < 0.0001$).

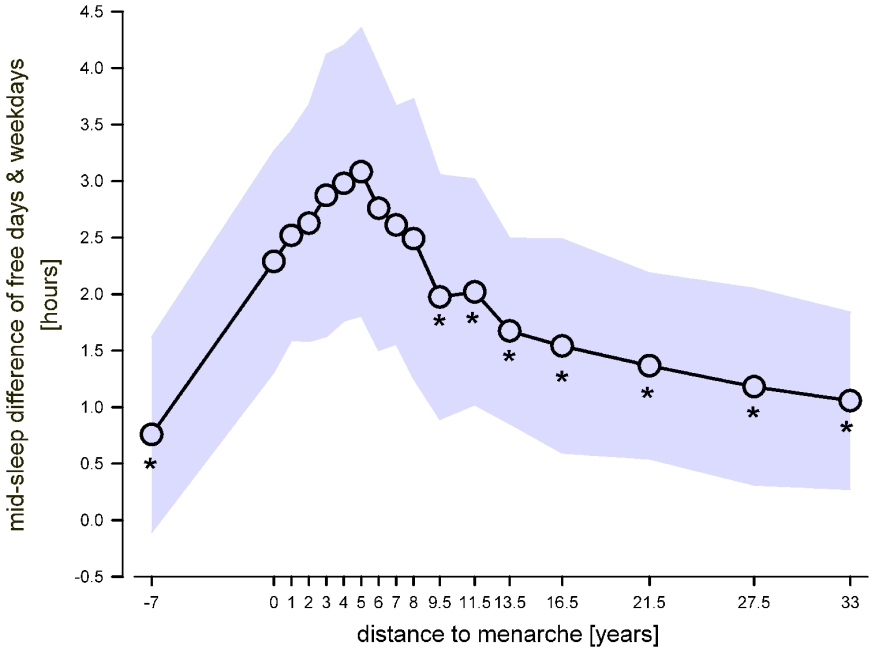

Figure 2.3.4: Difference in sleep midpoints between free days and weekdays with reference to pubertal maturation

*Mid-sleep represents the average difference between sleep phase midpoints on free days and sleep phase midpoints on weekdays. A striking switch-over from a consecutive delaying of the sleep midpoint difference to an advancing occurs 5 years after menarche. Shaded area represents ± SD; * indicate significant values compared to 5 years after menarche (Bonferroni adjusted alpha levels, for further statistics please see text).*

The average weekly sleep duration per day decreased remarkably from about 11 hours at prepuberty to less than 8 hours 2 years after menarche (Fig. 2.3.5). With progressive maturation (two years and more after menarche) average sleep duration levelled off between 7.5 and 8 hours. A Kruskal Wallis test yielded significance for the factor distance to menarche ($p < 0.001$).

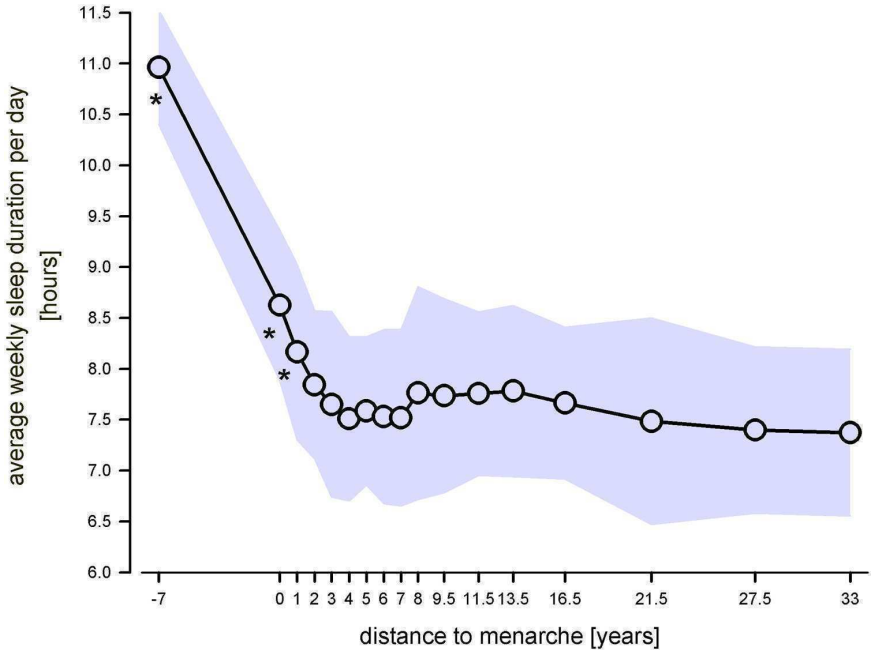

Figure 2.3.5: Average weekly sleep duration per day with reference to pubertal maturation

*Average weekly sleep duration per day reflects the average value calculated on the basis of 5 weekdays and 2 free days. Two years after menarche the average sleep duration per day remains remarkably stable between 7.5 and 8 hours for the remainder 'distance to menarche' classes. Shaded area represents ± SD; * indicate significant values compared to 5 years after menarche (Bonferroni adjusted alpha levels, for further statistics please see text).*

Post-hoc comparisons showed that only the average weekly sleep duration per day before the occurrence of menarche and 1 year after menarche was significantly different from the reference value at 5 years after menarche ($p < 0.003$). Furthermore, post-hoc comparisons revealed that after 2 years after menarche only values at 27.5 years and 33 years after menarche differed significantly from the value at 2 years after menarche ($p < 0.003$).

The highest sleep debt level occurred 1 year after menarche and amounted to almost 2 hours (Fig. 2.3.6). According to the Kruskal Wallis test the time course of the sleep debt with respect to distance to menarche was significant ($p < 0.001$). Post-hoc comparisons confirmed that sleep debt at 1 year after menarche was significantly different from the sleep debt before menarche and also from 6, 8 and more years after menarche ($p < 0.003$).

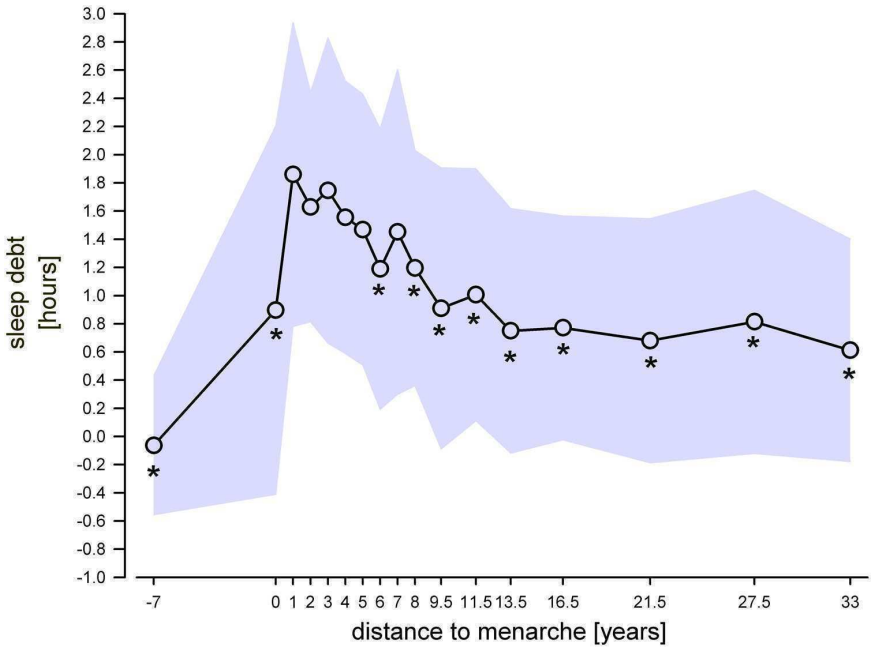

Figure 2.3.6: Sleep debt with reference to pubertal maturation

*Sleep debt represents the amount of sleep which is compensated for during free days due to a chronic sleep loss due to social demands during weekdays. The highest level of sleep debt occurs 1 year after menarche. Afterwards, a decrease in sleep debt was observed. Shaded area represents ± SD. * indicate significant values compared to 1 year after menarche (Bonferroni adjusted alpha levels, for further statistics please see text).*

2.4 Discussion

Changing psychosocial pressures during adolescence, such as increased evening leisure activities and earlier morning school schedules are important influences on sleep timing. Until now, the biological underpinnings of physiological sleep-wake cycles during maturation have been neglected. These regulatory processes seem to undergo realignment in phase relationships which may favour delayed sleep patterns in adolescents[134].

Our data, which show a distribution of chronotypes similar to an extensive sample mainly from Germany, Switzerland, and Austria illustrated in[235, 121], clearly point towards a temporal association between a biological marker of puberty and sleep phase preference during and after maturation. Moreover, our data provide evidence that the increased interference between individual sleep preference and social demands - as indexed by the difference of sleep midpoints on free and week-days - cannot be explained solely by an accumulation of a sleep debt after menarche lasting until 5 years thereafter. Thus, the difference in sleep duration between week and free days (or sleep debt as shown in Fig. 2.3.6) is relatively low before menarche, highest just after experiencing menarche, and decreases while the sleep phase preference is delaying until 5 years after menarche. However, on the basis of our

data, we cannot fully differentiate between social and biological influences on the evolution of sleep phase preference during maturation.

Studies have shown that as a consequence of social jetlag accumulated during the week (which increases on average up to 3 hours as shown here), there is an increased risk for mood dysregulation, impaired school performance, excessive daytime sleepiness, and addictive drug abuse in adolescents [235, 134, 245, 246, 247, 248, 249]. Furthermore, Knutson [250] reports that particularly females show a significant correlation between pubertal development and an increased risk of insomnia and waking tired. So far, chronobiological interventions to the circadian timing system, such as exposure of adolescents to bright light in the morning or scheduled naps, did not show any stabilising effect on the sleep-wake cycle or on performance [251, 252].

One important question about the underlying processes responsible for the switch to advance in sleep timing preference which occurs after 5 years after menarche remains to be examined. Is there a change in nocturnal plasma melatonin levels at this developmental stage other than the observed pubertal decline of this hormone [253, 254, 255]? It has been suggested that body size acts as mediator for the plasma melatonin decline during puberty[256]. Hence, does attainment of adult body size mediate a change in the circadian signal, which leads to the alteration in circadian phase preference? Our results provide evidence for a specific maturation-dependent time point to be considered in such in-depth analysis.

We conclude that the downsizing of social jetlag 5 years after menarche is a step towards adult-like behaviour as the ability to approach more aligned sleep-wake cycles during weekdays and weekends emerges. The distance to menarche may serve as an individual biological marker for the beginning of female adulthood. Thus, to answer the parents' question: the vibrant episode of adolescence should be over 5 years after menarche.

Acknowledgements

We are grateful to the high schools and University involved for supporting the current survey and the subjects for participating. We thank Anna Wirz-Justice for her precious and helpful comments. Our thanks are also due to Giovanni Balestrieri for data processing assistance.

Supporting material

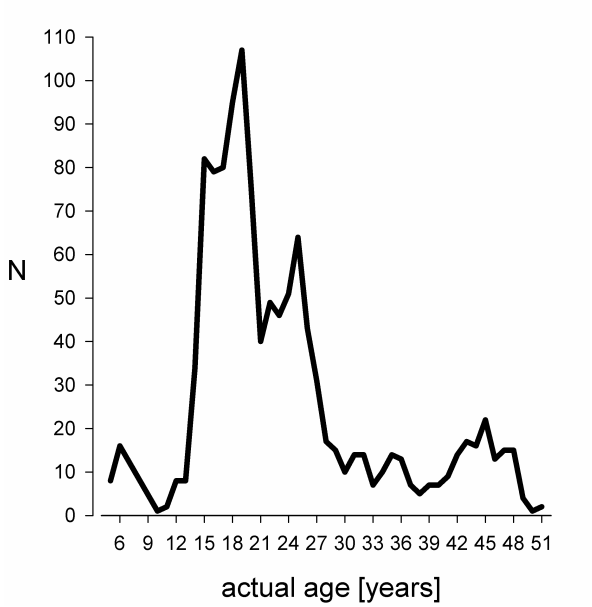

Figure S1: Age distribution within the study sample

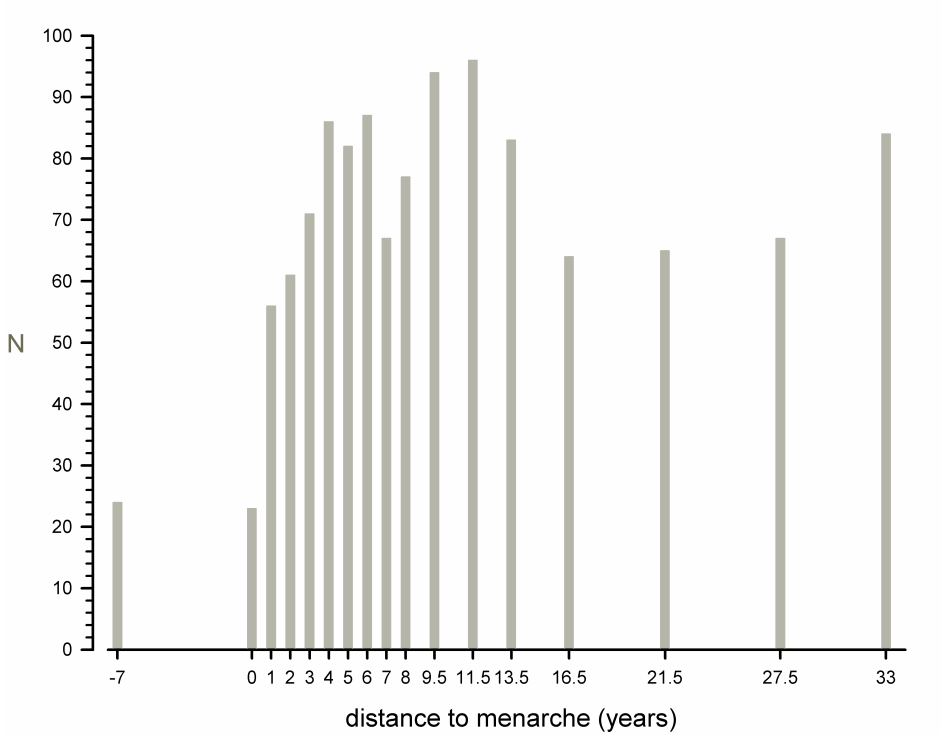

Figure S2: Sample distribution with respect to distance to menarche

Distance to menarche represents the difference between actual age and the age at the occurrence of menarche. It serves as indirect measure for pubertal maturation. A distance of -7 years to menarche means that the females in this group did not yet experience menarche. Furthermore, it means that the temporal distance to this event is 7 years based on the actual mean age of the subjects of this group compared with the median age at occurrence of menarche calculated from the sample.

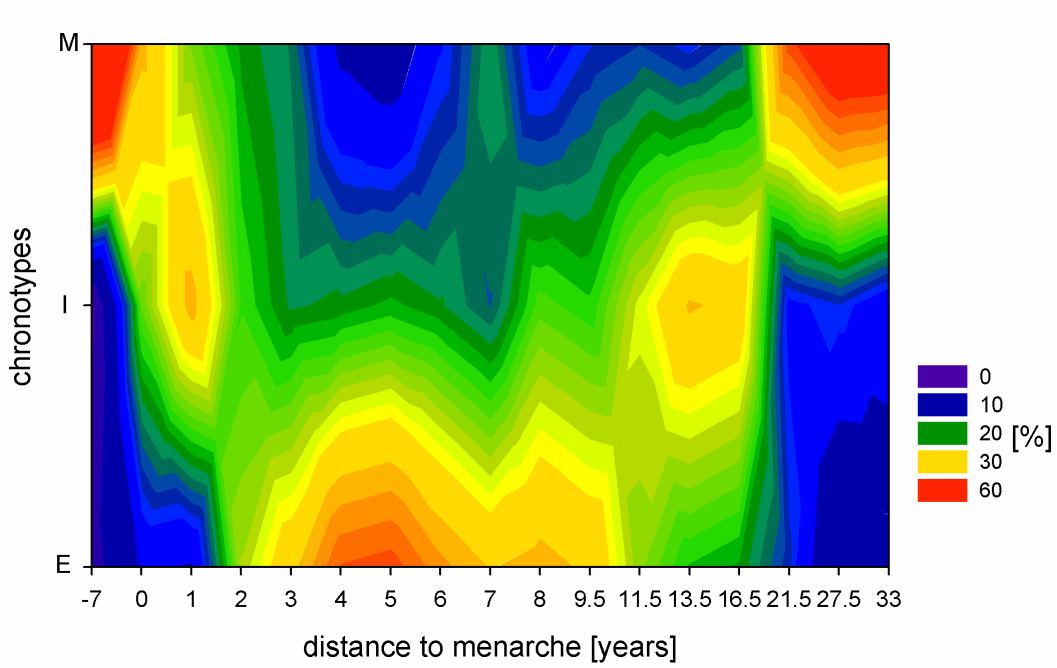

Figure S3: Chronotype distribution with reference to pubertal maturation

The contour plot represents the distribution of the different chronotypes occurring at each considered time point of pubertal maturation (i.e. distance to menarche). Different colours between the contour curves indicate the magnitude of the percent portion of the chronotypes at a specific time point of pubertal maturation as indexed beside the figure. Chronotype abbreviations: E= evening type, I =intermediate type, M= morning type.

3 Challenging the sleep homeostat in young depressed and healthy older women: sleep in depression is not premature aging

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Abstract

Major depression and sleep disturbances are closely related and often occur concomitantly. This led to the hypothesis of a deficiency in homeostatic sleep pressure in depression (S-deficiency hypothesis). Many of the observed changes of sleep characteristics in depression are also present in healthy aging, which led to the premise that sleep in depression resembles premature aging. Here, we aimed at quantifying the homeostatic and circadian sleep-wake regulatory components in young women suffering from major depressive disorder and healthy young and older control women during 40 hours of sustained wakefulness under constant routine conditions.

After an 8-h baseline night nine depressed women, eight healthy young and eight healthy older women underwent a 40-hour sustained wakefulness protocol followed by a recovery night under constant environmental conditions. Polysomnographic recordings were carried out continuously and subjective sleepiness and mood was assessed half-hourly along with salivary melatonin, as a circadian marker throughout the 40-h protocol. Sleep parameters as well as NREM sleep EEG spectra in the frequency range of 0.75-25 Hz along the anterio-posterior axis were analysed during the baseline and the recovery sleep episode. In particular, the time course of EEG slow-wave activity (SWA) (EEG spectra range: 0.75-4.5 Hz), as a marker of homeostatic sleep pressure, was analysed during the recovery night in order to assess homeostatic response to sleep deprivation.

All three groups showed the typical increase of SWA during the first part of the recovery night as a response to increased homeostatic sleep pressure. In general, young depressed women exhibited higher absolute mean SWA levels and a stronger response to sleep deprivation in the delta frequency range compared to healthy young and healthy older women, particularly in frontal brain regions. In contrast, healthy older women exhibited not only attenuated SWA values compared to the other two groups but also an absence of the frontal predominance of mean SWA during the recovery night. Relative EEG spectra also showed higher homeostatic response to sleep deprivation in young depressed compared to young and older healthy volunteers, particularly in the first sleep cycle but also in the second sleep cycle when compared to healthy young. Furthermore, nighttime melatonin secretion was reduced in depressed and older women compared to young women.

Our data clearly show that homeostatic sleep regulation as well as sleep architecture in young depressed women is not equal to premature aging. Moreover, our findings demonstrate that young depressed women without sleep disturbances exhibit no deficiency in the sleep homeostatic process S but rather live on an elevated level of homeostatic sleep pressure. We hypothesise that a reduced circadian arousal signal during wakefulness may contribute to this homeostatic overexpression.

3.1 Introduction

According to the World Health Organization (WHO) depression holds the 2nd rank of diseases causing loss of productive life in the age category between 15 and 59 years worldwide [138]. Despite its high prevalence and socioeconomic impact as well as the considerable research efforts during the past decades, the knowledge on the aetiology and pathophysiology of major depression remains quite fragmented [149, 150, 151]. However, epidemiological studies have shown that vulnerability to depression is gender and age dependent with a twice as high risk in women compared to men during the reproductive years [142, 143, 145, 144]. Furthermore, environmental factors such as stress, emotional trauma and viral infections and their interaction with a genetic predisposition have been shown to play a pivotal role in the development of the illness [152, 157, 257, 258, 153, 155, 158].

Clinical observations and polysomnographic recordings show that major depression is often associated with sleep disturbances although sleep disturbances are neither depression-specific nor a compulsory symptom for the clinical diagnosis of the illness [159]. As a result of the current understanding of the importance of the circadian system and sleep-wake homeostasis in sleep-wake regulation, these two processes have been suggested to be crucially involved in the pathogenesis of major depression [186]. Reports on sleep disturbances in depression comprise longer sleep latencies, shortened rapid-eye-movement sleep (REM) latency, increased REM sleep in the beginning of the night, higher wake-up tendency in the latter part of the night, early termination of sleep in the morning, decreased slow-wave sleep (SWS) and electroencephalographic (EEG) slow-wave activity (SWA, EEG power density between 0.75-4.5 Hz) [259, 199]. However, the scientific findings on sleep disturbances are not consistent throughout literature as various studies failed to demonstrate or confirm such changes [204, 205, 206, 207]. This inconsistency is mainly due to differences in age, sex, clinical characteristic and subtype of depression, severity of depression as well as to the heterogeneity of the applied study settings [195, 260, 196, 261, 203, 262, 263, 161, 216].

According to the two-process model of sleep regulation the interaction between a sleep-wake dependent process S (= sleep-wake homeostat) and a circadian process C is responsible for the timing of sleep and wakefulness [53, 51, 52]. The findings that manipulation of the sleep-wake cycle (e.g. sleep deprivation, slow-wave sleep deprivation, sleep phase advance etc.) and/or circadian phase (e.g. timed light therapy) influence the course of depression, gave rise to several hypothesis relating the involvement of processes controlled by the circadian pacemaker, the sleep-wake homeostat, or the interaction between both [189, 211, 216, 109, 53, 113]. These hypotheses comprise the so-called S-deficiency hypothesis [113], the phase-advance hypothesis [108], and the acetylcholine-monoamine imbalance hypothesis [264]. While the latter two mainly deal with changes in the circadian or ultradian timing the S-deficiency hypothesis refers to homeostatic alterations of the regulation of sleep. It states that sleep disturbances in patients with major depressive disorder (MDD) reflect a deficiency in the homeostatic buildup of sleep pressure during wakefulness [113].

Frontal delta EEG activity during NREM sleep is a physiological measure of the dissipation of process S during sleep and of the accumulated need for sleep during wakefulness respectively [82, 81]. Its level is positively correlated with the amount of time spent awake [39]. Thus, within the framework of the S-deficiency hypothesis, MDD show a significant reduced response to a challenge of the sleep-wake homeostat (i.e. sleep deprivation), as indexed by EEG delta activity during recovery sleep. So far two studies support the S-deficiency hypothesis in major depression [112, 265], whereas one study added refinements such as a sex-dependency of the S-deficiency in depression with only men exhibiting lower EEG delta activities during sleep [266], and another study reported no S-deficiency in untreated middle-aged depressive outpatients compared to controls [267].

With age, different alterations of sleep parameters occur such as increased sleep fragmentation, a reduction of SWS and SWA, and a reduced frontal EEG response to sleep deprivation [197, 94, 97]. Although these changes are typical signs of healthy aging, it is striking how similar they are to the sleep abnormalities reported in MDD. Thus, with regard to sleep, it has been argued that depression has similarities to precocious ageing [195]. A refinement of this consideration has been suggested as such that depression might bear sleep-related similarities to premature ageing only for restricted sleep characteristics such as sleep efficiency, total sleep time, intermittent time awake during sleep, and REM sleep latency [196].

A dissection of the contributions of circadian and homeostatic processes to sleep-wake regulation can only be assessed by applying specific sleep-wake manipulation schemes such as the forced desynchrony protocol or the constant routine protocol [224]. While the forced desynchrony protocol allows seperating the influence of the sleep-wake homeostat and the circadian timing system [55], the constant routine protocol is mainly designed to unmask endogenous circadian rhythms by controlling for environmental conditions such as light, food intake, body posture, physical activity, and sleep [224]. Study participants of constant routine protocols usually remain awake for 24 up to 60 hours, which leads to a progressive increase in homeostatic sleep drive while at the same time endogenous circadian phase is changing. This setting allows to analyze homeostatic sleep regulation after sleep deprivation and to assess phase and amplitude of unmasked circadian rhythms such as of melatonin or core body temperature. To our knowledge, there are only a few studies, in which the homeostatic EEG SWA response to sleep deprivation in depression has been investigated [185, 267**?**] and there is no study so far on homeostatic sleep regulation in depression under constant routine conditions. Here we aimed at investigating the sleep architecture and homeostatic sleep regulation during NREM sleep of unmedicated young women with non-seasonal major depressive disorder under high sleep pressure during a 40-h constant routine protocol compared to age and sex matched healthy young and to sex matched healthy older controls. Based on the S-deficiency and the premature aging premise we hypothesized an attenuated homeostatic response to sleep deprivation, as indexed by a reduced EEG delta activity during NREM sleep in young depressed when compared to young healthy women but not to older healthy women. Second, we expected a decreased frontal predominance of EEG delta activity in young depressed compared to healthy young women.

3.2 Methods

3.2.1 Study participants

Eight healthy young (HY, 20-31y, mean = 25.4y +/- SD 3.8y), eight healthy older (HO, 57-74y, mean age = 64.1y +/- SD 5.5y), and nine young women with major depressive disorder (MDD, 20-32y, mean age = 26.2y +/- SD 5.2y) participated in the study. A two sided t-test disclosed no significant age differences between MDD and HY. As a matter of fact, the age difference between the young and older cohort was significant (t-test; $p < 0.001$).

Study participants were recruited by advertisements at the Universities in the region of Basel, Switzerland and through selected online portals. All participants underwent a defined screening procedure which included questionnaires referring to physical health, drug consumption, and sleep quality as well as a medical examination to assess somatic state. The screening for the MDD participants included additional self-reported depression ratings with the Beck Depression Inventory (BDI) [268] whereby only participants with a score >12 were considered for the subsequent clinical interview (mean BDI value = 20.2 ± SD 9.7). To assess the presence of a major depressive disorder a structured clinical interview for DSM-IV Axis I (SCID-I) according to the diagnostic and statistical manual of the American Psychiatric Association (DMS-IV_R)[159] was carried out with the respective MDD volunteers (mean SCID-I value = 5.1 ± SD 0.3). The MDD participants had no atypical symptoms and no psychiatric comorbidity according to DSM-IV_R.

Among the healthy subjects, only participants with no sleep disturbances as assessed by the Pittsburgh Sleep Quality Index (PSQI) [13]) were included in the study (PSQI value ≤ 5) whereas for MDD participants a score ≤ 8 was allowed (i.e. mild forms of sleep disturbances) (MDD = 6.1 ± 1.7, HY = 2 ± 1.7, HO = 3.8 ± 1.7, mean +/- SD; t-test: MDD > HY $p < 0.001$ and MDD > HO $p < 0.05$). Additionally, sleep disorders were excluded based on recordings during an adaptation night in the chronobiology laboratory. Applied exclusion criteria included a sleep efficiency of less than 80%, more than 10 periodic leg movements/hour, and an apnea-hypopnoea index > 10. Medications other than oral contraceptives were not allowed for all participants, who were drug-free (verified by urinary toxicological analysis), nonsmokers, and had no shift work or flights over more than 3 time zones during the last 3 months before the study began. Only intermediate chronotypes as assessed by the diurnal type scale [11] were considered (MDD = 15.9 ± 1.3, HY = 15.6 ± 3.8, HO = 18 ± 3.4; mean ± SD; t-test $p > 0.05$). All women participated in the laboratory phase of the study during their follicular phase of their menstrual cycle (days 1-5 after menses onset) and gave their signed informed consent for the participation in the study. The study procedures as well as all questionnaires and the consent form were approved by the local Ethics Committee of Basle (EKBB), Switzerland, and all procedures conformed to the Declaration of Helsinki.

3.2.2 Study protocol

The study comprised an ambulatory part at home (1 week) followed by a laboratory part (3.5 days). During the ambulatory part volunteers were asked to restrict caffeine intake to only one beverage per day, to drink not more than 5 alcoholic drinks during the entire week, and to abstain from heavy physical exercises. Furthermore, they were asked to keep a regular sleep-wake schedule during the ambulatory part of the study. Compliance was verified by sleep logs and ambulatory activity measurements by means of a wrist activity monitor (Cambridge Neurotechnology Ltd®, UK). The timing of the sleep-wake schedule during the protocol was adjusted to individual habitual bedtimes calculated by centering the approximate 8-hour sleep episodes during the baseline week at the individual midpoint of sleep of each participant. Habitual bedtimes between healthy young (11:52 PM ± SD 69 min) and young depressed (11:53 PM ± SD 50 min) as well as between healthy young and older women (11:05 PM ± SD 31 min) were not significantly different ($p > 0.9$ and $p > 0.1$ respectively) but young depressed differed significantly from healthy older women ($p < 0.05$; t-test for independent samples). The protocol comprised a habituation night followed by a baseline night in the chronobiology laboratory. The baseline night was followed by 40-hours of sustained wakefulness and an 8-h recovery night (Fig. 3.2.1).

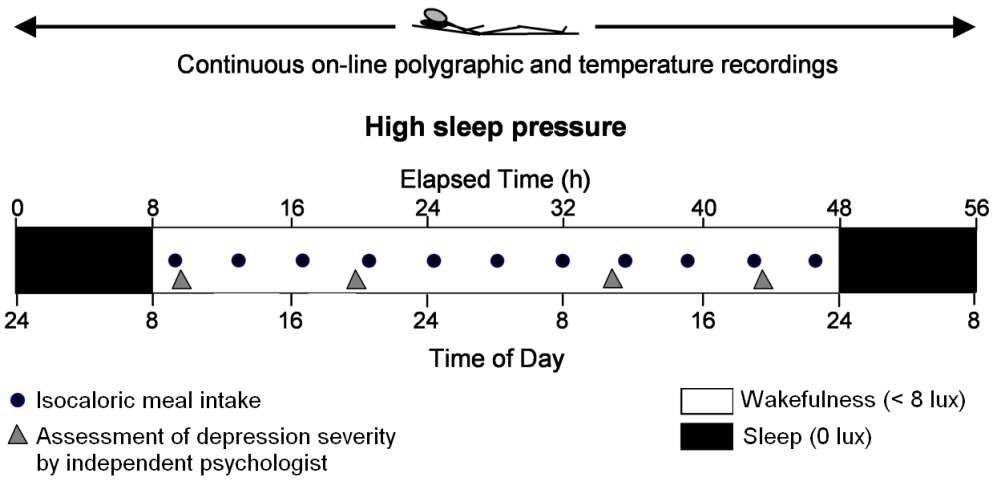

Figure 3.2.1: Schematic overview of the study protocol

The illustration shows the timing of scheduled sleep and wakefulness, food intake and psychological assessments in the course of the sustained wakefulness (SW) protocol. Due to 40 hours of sustained wakefulness the SW protocol challenges the sleep homeostat leading to high sleep pressure levels. In total, bedrest conditions lasted 56 hours (including baseline and recovery nights) whereby the timing of sleep and wakefulness, body posture, light levels, food intake, and environmental temperature were controlled for (constant routine conditions).

EEG recordings started in the afternoon after the habituation night. During the laboratory part participants remained under constant conditions such as dim-light < 8 lux during

scheduled wakefulness and 0 lux during scheduled sleep episodes, semirecumbent posture position in bed during wakefulness, regular isocaloric meals, and constant room temperature (for details on the study protocol see Fig. 3.2.1). A daily heparin injection was given to the healthy older women in order to prevent venous thrombosis (Fragmin®, 0.2 mL, 2500 IE/UI, Pfizer AG, Switzerland). The severity of the depressive episode of young volunteers with major depression was assessed regularly (see Fig. 3.2.1) by an independent psychologist on the basis of the Montgomery-Asberg Depression Rating Scale (MADRS) [269] and the Hamilton Rating Scale for Depression (HAMD-17) [270].

3.2.3 Polysomnographic recordings and analysis

Polysomnographic recordings (Vitaport-3 digital recorder TEMEC Instruments BV, Kerkrade, the Netherlands) during sleep comprised twelve EEG derivations (F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, O1, O2, Oz referenced against linked mastoids), two electrooculograms, one submental electromyogram, and one electrocardiogram. All EEG signals were filtered at 30 Hz (fourth-order Bessel-type antialiasing low-pass filter, total 24 dB/Oct), and a time constant of 1.0 second was used prior to online digitization (range 610 µV, 12 bit AD converter, 0.15 µV/bit; storage sampling rate at 128 Hz). Sleep episodes were visually scored on a 20-s epoch basis according to the standard criteria of Rechtschaffen and Kales [29]. EEGs were subjected to spectral analysis using a fast Fourier transform (FFT) with 10% cosine 4-second windows resulting in a 0.25 Hz resolution. Sleep stages (1-4), rapid eye movement (REM) sleep, movement time (MT) were expressed as percentage of total sleep time (TST) during the respective night for all participants. TST and sleep latencies to stage 1 (SL1), stage 2 (SL2), to REM (RL) were indicated in minutes. Sleep efficiency (SE) was defined as follows: SE = TST/time between lights off and lights on (TIB) x 100. Wakefulness after lights off (WALO; % of TIB) and wakefulness after sleep onset (WASO; % of TST) were also measured. Non-rapid eye movement (NREM) sleep was defined as stages 2 to 4 (% of TST).

EEG spectra during the baseline and recovery nights were calculated for the first 7 hours of sleep in the frequency range from 0.75 to 25 Hz for all 12 derivations. A mixed-model 4-way rAnova for the factors group (young depressed vs healthy young vs healthy older women), night (baseline vs recovery), derivation site (frontal vs central vs parietal vs occipital) and hemisphere (left vs right side derivations) disclosed significant lower left hemisphere EEG values for one frequency bin in the delta range (0.75 Hz) and one bin in the theta range (6.5 Hz) ($p < 0.05$). No further significancies of single factors nor of interactions of the factors group*hemisphere, derivation site*hemisphere, night*hemisphere, group*hemisphere*derivation site, and group*hemisphere*derivation site*night were observed (p at least > 0.1, Tukey-Kramer adjusted p values). Hence, spectral values for side and midline derivations were collapsed along the anterior-posterior axis resulting in one value for each of the F, C, P, and O sites. Baseline and recovery night spectral values of young depressed and healthy older women were graphically illustrated as percentage of the

respective values of the healthy young group during baseline and recovery night whereby the statistical analyses were based on absolute values. Group differences in EEG power density were assessed by mixed-model repeated-measures ANOVA (rANOVA).

NREM/REM sleep cycles were defined according to the criteria of Feinberg and Floyd[271] with the exception that, for the last sleep cycle, no minimum REM-sleep duration was required. Thereafter, each sleep cycle was divided into 10 equal time intervals during NREM sleep and 4 equal time intervals during REM sleep.

To investigate the decay of SWA (0.75-4.5 Hz) during NREM sleep, being a physiological correlate of the decrease of process S, across the baseline and the recovery nights the following exponential decay function was fitted to the data of each group of participants and all NREM cycles: $SWA_t = SWA_\infty + SWA_0 * e(-rt)$; with SWA_t = averaged SWA per sleep cycle, SWA_0 = intercept on the y axis, SWA_∞= horizontal asymptote for time $t = \infty$, r = slope of the decay, t = average timing of the NREM cycle midpoint.

A topographic, colour coded illustration of mean SWA during the baseline and recovery night (see Fig. 3.3.6) was prepared by VPDReader, a software specifically designed for this purpose by M. Hofstetter, Centre for Chronobiology, Basel.

3.2.4 Salivary melatonin sampling

Saliva collections were scheduled every 30 minutes throughout the entire 40-h sustained wakefulness protocol. A direct double-antibody radioimmunoassay (RIA) was used for the melatonin assay which was validated by gas chromatography–mass spectroscopy with an analytical least detectable dose of 0.65 pm/ml (Bühlmann Laboratories, Schönenbuch, Switzerland)[272]. All melatonin values were collapsed into 2.5-h bins per subject before averaging over subjects whereby missing values were linearly interpolated.

3.2.5 Subjective mood and sleepiness ratings

Study participants rated their sleepiness and mood at the same time intervals as melatonin samples were taken (every 30 min over the entire 40-h protocol). Mood ratings were measured by the visual analogue scale (VAS)[273] with values from 0 mm (depressed mood) to 100 mm (good mood) and sleepiness was assessed by the Karolinska Sleepiness Scale (KSS) with a rating range from 1 (not sleepy) to 9 (very sleepy) [274]. KSS and VAS values were collapsed into 2.5-h bins per subject before averaging over subjects.

3.2.6 Statistical analysis

The statistical packages SAS ® (SAS Institute, Inc.; Version 9.1.3) and Statistica ® (StatSoft Inc., STATISTICA for windows, Version 8.0) were used. Sleep stages were analysed

with repeated-measures ANOVA (PROC GLM) and p values were based on Huynh-Feldt corrected degrees of freedom. Post-hoc comparisons on sleep stages were based on Duncan's multiple range test and the levels of significance of these posthoc comparisons were adjusted according to the false discovery rate procedure [275]. Comparisons of EEG spectra, melatonin, subjective sleepiness and mood between groups were done with mixed-model repeated-measures ANOVA (PROC MIXED) and p values were based on Kenward-Roger's corrected degrees of freedom [276]. Contrasts were assessed with the LSMEANS statement and the respective level of significance was adjusted according to the Tukey-Kramer method [277]. 1-, 2-, 3- and 4-way mixed-model rANOVAs were used with the categorical factor group (MDD vs HY vs HO) and the repetitive factors derivation, night, and time interval (e.g. sleep cycle etc.).

3.3 Results

3.3.1 Sleep stages during baseline and recovery nights

Sleep parameters for the baseline and recovery nights based on visual scoring for the young depressed (MDD), healthy young (HY), and healthy older women (HO) are summarized in Table 3.1. A 2-way rAnova disclosed significant effects of the main factor group for total sleep time (TST, $p < 0.01$), sleep efficiency (SE, $p < 0.01$), wake after lights off (WALO, $p < 0.001$), wake after sleep onset (WASO, $p < 0.001$), and stage 4 sleep ($p < 0.05$). Post-hoc analysis on significant effects of the factor group showed significantly less TST ($p < 0.01$), lower SE ($p < 0.01$), and less stage 4 sleep ($p < 0.05$) of HO compared to MDD and HY. Furthermore, HO had significant longer wake durations after lights off and after sleep onset than MDD and HY ($p < 0.01$ and $p < 0.001$ respectively) and a tendency to less REM sleep compared to HY ($p < 0.01$). No significant differences between young depressed and healthy young women were observed for any of the sleep parameters (p at least > 0.01).

The 40-h of sustained wakefulness resulted in significant alteration in sleep architecture during the recovery night compared to baseline night for all three groups in the following parameters: higher TST ($p < 0.01$), increase in SE, decrease in wake times after lights off, lower sleep latency to stage 2 sleep (SL2) ($p < 0.05$), less stage 1 and stage 2 sleep, more stage 3 and stage 4 sleep as well as more slow-wave sleep (SWS) and NREM sleep ($p < 0.001$).

For stage 4 sleep, a 2-way rAnova yielded a significant interaction between group*night ($p < 0.05$) with higher values during the recovery night compared to baseline night in all three groups ($p<0.001$) and significant higher values of MDD and HY during the recovery night than HO ($p < 0.01$ and $p < 0.05$, respectively).

Table 3.1: Sleep parameters of the baseline and recovery nights based on visual scoring for all groups

Parameter	young depressed women		healthy young women		healthy older women		2-way rAnova					
	baseline	recovery	baseline	recovery	baseline	recovery	group		night		group x night	
							p	$F_{2,22}$	p	$F_{1,22}$	p	$F_{2,22}$
TST, min	444.4 ± 24	459.7 ± 15.1	446.2 ± 26.9	463.6 ± 13.2	408.5 ± 42.5	434.1 ± 31.2	**	5.86	**	9.43	-	0.25
SE, %	94.4 ± 1.8	95.7 ± 3.1	93.0 ± 5.6	96.7 ± 2.8	85.1 ± 8.8	90.4 ± 6.5	**	7.8	*	7.7	-	0.9
WALO, %	3.9 ± 1.7	2.8 ± 3	4.1 ± 4.4	1.0 ± 0.8	12.9 ± 8.3	8.1 ± 6.1	***	11.35	*	6.44	-	0.84
WASO, %	1.9 ± 1.3	1.4 ± 1.8	3.0 ± 4.8	0.6 ± 0.9	14.1 ± 11.7	7.5 ± 6.6	***	12.04	°	4.08	-	1.38
SL1, min	9.9 ± 7.4	6.9 ± 6.7	8.6 ± 9	2.6 ± 1.3	8.5 ± 3.1	8.7 ± 9.1	-	0.76	°	3.51	-	1.26
SL2, min	12.7 ± 7.7	9.9 ± 8	14.1 ± 9.2	5.4 ± 1.8	11.1 ± 4.3	11.1 ± 8.2	-	0.16	*	5.93	°	2.64
RL, min	96.1 ± 31	85.8 ± 44.3	79.6 ± 30.8	72.9 ± 40.2	120.4 ± 104.8	80 ± 34.9	-	0.92	-	1.42	-	0.43
MT, %	0.6 ± 0.2	0.5 ± 0.2	0.9 ± 0.5	0.8 ± 0.7	0.8 ± 0.6	0.5 ± 0.3	-	1.35	°	5.1	-	0.36
St1, %	8.5 ± 3.3	5.3 ± 4.1	10.9 ± 3.4	5.2 ± 2.4	11.8 ± 5	8.7 ± 2.9	-	2.22	***	51.59	-	2.38
St2, %	54.2 ± 5.9	50.1 ± 6.3	50.2 ± 5.7	46.1 ± 5.2	58.7 ± 13.9	54.8 ± 13.6	-	1.92	***	17.02	-	0
St3, %	10.4 ± 2.9	11.4 ± 3.5	11.1 ± 2.8	13.2 ± 4.2	11.3 ± 5.3	15.0 ± 4.4	-	0.83	***	14.6	-	1.7
St4,%	8.1 ± 5.9	16.8 ± 9.6	7.5 ± 6.1	15.1 ± 6	2.8 ± 2.5	6.4 ± 4.4	*	4.19	***	69.71	*	3.83
SWS, %	18.4 ± 6.2	28.2 ± 9.3	18.7 ± 7.5	28.3 ± 6.7	14.1 ± 7.5	21.4 ± 8.5	-	1.59	***	109.89	-	0.87
NREM, %	72.7 ± 5.9	78.3 ± 7.1	68.9 ± 4.5	74.4 ± 4.1	72.8 ± 8.9	76.2 ± 7.8	-	0.83	***	38.46	-	0.88
REM, %	18.9 ± 3.8	16.4 ± 4.6	20.2 ± 2.3	20.4 ± 3.7	15.4 ± 6.4	15.1 ± 6.1	°	3.11	-	0.88	-	0.84

Sleep parameters are averaged separately across the baseline night and the recovery night (mean ± SD) for young women with major depression (n=9), young healthy women (n=8), and older healthy women (n=8). TST = total sleep time; SE = sleep efficiency (TST / TIB * 100); WALO = wake after lights off (in % of total time in bed between lights off and lights on); WASO = wake after sleep onset (in % of TST); SL1 = sleep latency to stage 1; SL2 = sleep latency to stage 2; RL = REM latency; MT = movement time after sleep onset (in % of TST); St1-St4 = sleep stages 1-4 (in % of TST); SWS = slow-wave sleep (sum of stages 3 and 4 in % of TST); NREM = non-REM sleep (sum of sleep stages 2-4 in % of TST); REM = REM sleep in % of TST; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, °$p < 0.1$.

3.3.2 EEG power density during the baseline and recovery nights

Relative EEG spectra with reference to healthy young (100%) during NREM sleep for the baseline and recovery nights in the frequency range between 0.75 and 25 Hz are illustrated in Figure 3.3.1. Mixed-model rANOVA disclosed significant higher mean EEG power of young depressed (MDD) than healthy older (HO) women for the delta frequency bins 1-3 Hz ($p < 0.05$).

EEG power during recovery night was significantly higher than during baseline night for all groups for the frequency bins of the delta, theta and alpha range (0.75-10.5 Hz, $p < 0.001$; 10.75 Hz, $p < 0.01$; 11 Hz, $p < 0.05$; mixed-model 3-way rANOVA) as well as in most of the beta frequency bins (16.75-17 Hz, $p < 0.05$; 17.25-25 Hz, $p < 0.01$). Significant interaction of the factors group*night occurred in the delta frequency bins 1-4 Hz and in some of the theta frequency bins (6.25-6.5 Hz, 7.25-7.75 Hz) with higher values during recovery night than baseline night in MDD for all respective frequency bins (post-hoc comparisons according to LSMEANS statement, Tukey-Kramer adjusted; $p < 0.001$), in HY in the same range

except the frequency bins 7.5-7.75 Hz ($p < 0.01$), and in HO in some of the delta and theta frequency bins (2-4 Hz, 6-25-6.5 Hz, 7.25 Hz). Post-hoc comparison showed furthermore higher values for MDDs than HO in the delta frequency bins 1-3.25 Hz ($p < 0.05$) and for HY than HO in the delta frequency bins 1.5-1.75 Hz ($p < 0.05$) during recovery night.

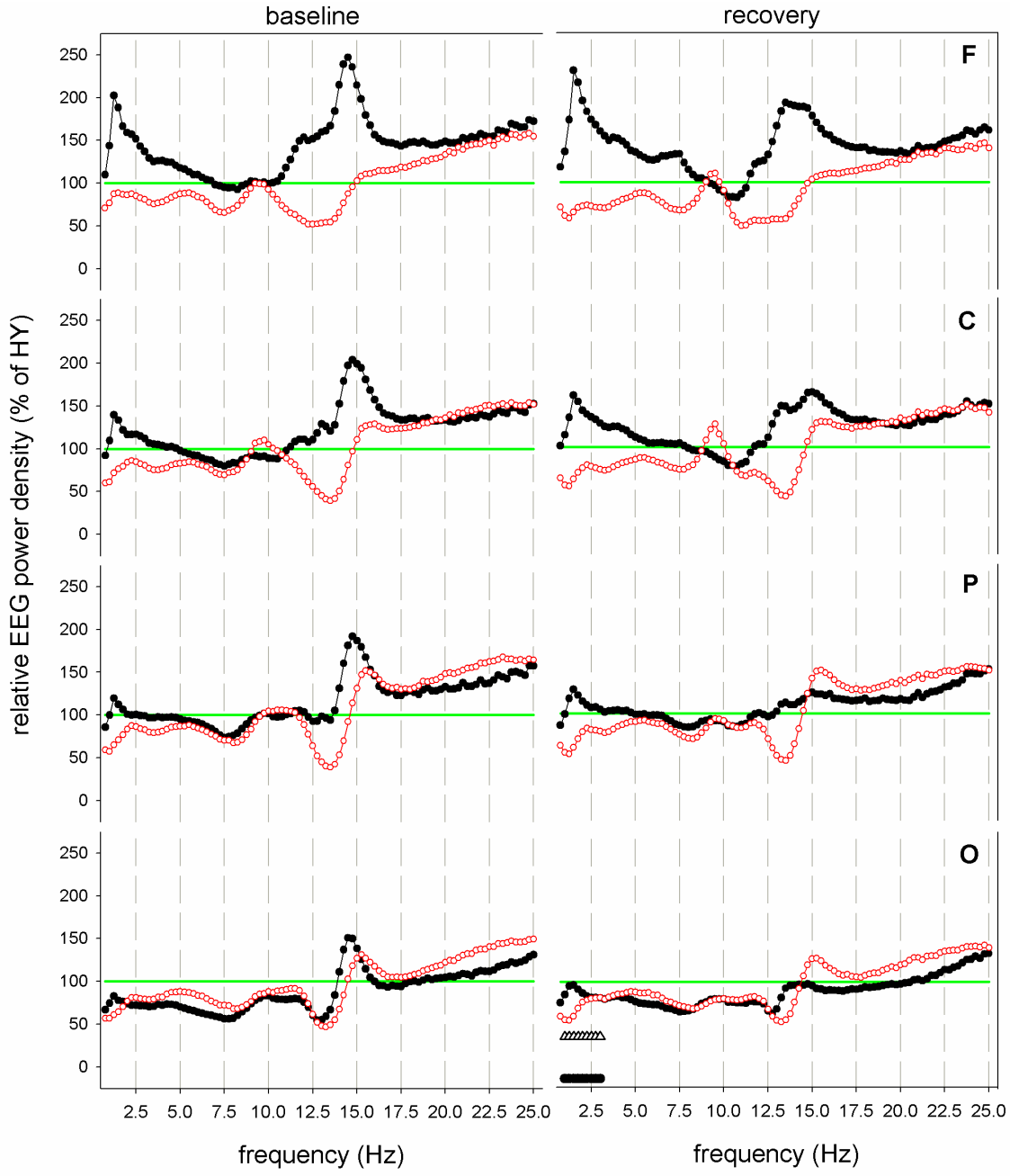

Figure 3.3.1: Relative EEG spectra during baseline and recovery nights (expressed in % of HY)

Relative EEG spectra values for baseline and recovery nights with reference to respective levels of healthy young (reference line at 100%; n=8) are shown for healthy older (HO; red open circles; n=8), and young depressed (MDD; black circles; n=9) women between 0.75-25 Hz for F, C, P, and O derivations (collapsed left, central, and right values along the anterior posterior axis). Significant differences between the groups are indicated near the abscissae as well as respective post-hoc comparisons (black circles = significant group effect; open triangles = MDD vs HO; $p < 0.05$; analysis based on absolute EEG spectra values).

Mixed-model rANOVA disclosed significant effects of the main factor derivation in all frequency bins except some of the theta range (6-7 Hz) with highest values in the frontal (F) derivations ($p < 0.05$). Furthermore, the analysis showed significant interaction of the factors group*derivation in all frequency bins of the delta and theta range (0.75-8 Hz, $p < 0.001$) as well as in some of the frequency bins of the alpha, sigma, and beta range (11.25-14 Hz and 14.75-25 Hz). Post-hoc comparisons showed higher values for MDD than HY and HO for the F derivations in bins of the delta frequency range (1.25-3 Hz, MDD > HO $p < 0.001$, MDD > HY $p < 0.05$) and higher values for MDD than HO in the delta frequency range (3.5-4.25 Hz, $p < 0.05$) and in the alpha/sigma range (11.5-12.5 Hz, $p < 0.05$). Finally, mixed-model 3-way rAnova disclosed significant interactions of the factors group*night*derivation for some of the frequency bins of the delta and theta range (0.75-5 Hz and 6.5-7.5 Hz, $p < 0.05$) with higher values of MDD than HY and HO in the F derivation during recovery night in the delta frequency bins 1.5-3 Hz ($p < 0.05$).

Relative EEG spectra per sleep cycle (recovery night cycle spectra in % of baseline values per cycle) during NREM sleep for the frequency range 0.75-25 Hz for F, C, P, and O derivations were calculated in order to highlight EEG power modulation over the night in more detail (Figure 3.3.2). Because mixed-model 3-way rANOVA with the factors group, cycle, and derivation showed significant interaction only in three frequency bins 7.25-7.75 Hz ($p < 0.05$) statistical analyses was performed for each cycle separately.

Mixed-model 2-way rAnova with the factors group and derivation yielded significance for the factor group in some of the delta frequency bins in cycle 1 (1.5-2 Hz and 2.75-4.5 Hz; $p < 0.05$) and in cycle 2 (2.75 Hz and 3.25-4.25 Hz; $p < 0.05$). Furthermore, EEG power values differed also significantly between groups in the theta/alpha frequency bins (4.75-8.25 Hz) and in some of the sigma frequency bins (13.5 Hz, 14-14.25 Hz) during cycle 1 ($p < 0.05$), in the theta frequency range during cycle 2 (6.25 Hz, 7-7.5 Hz; $p < 0.05$), and in the theta/alpha frequency range (7.75-8.25 Hz and 8.75 Hz; $p < 0.05$) during cycle 3. Post-hoc comparisons showed thereby for cycle 1 higher values of young depressed (MDD) compared to healthy older (HO) women for the frequency bins 1.5-2 Hz and 2.75-7.75 Hz ($p < 0.01$) as well as higher values of MDDs compared to HY in the delta range (3.75-4 Hz; $p = 0.05$), in the theta frequency bins 4.5-4.75 Hz and in the alpha frequency bins 5.25-8.25 Hz, and in some bins of the sigma frequency range (13 Hz and 14-14.25 Hz) ($p < 0.05$; LSMEANS procedure, Tukey-Kramer adjusted). During cycle 2 MDD had significantly higher relative EEG power than healthy young women in some frequency bins of the delta range (3.75-4.25 Hz) and in some theta frequency bins (6.25 Hz and 7-7.5 Hz, $p < 0.05$). During cycle 3, young depressed women had higher relative spectra values than HO in one of the theta frequency bins (7.75 Hz; $p < 0.05$).

Significant interaction of the factors group*derivation was observed in some frequency bins of the delta range (1.5-1.75 Hz and 2.75 Hz) and in the theta range (7.25-7.5 Hz) during cycle 1, in the delta frequency bins 1-2.75 Hz during cycle 2 as well as in the frequency bins 1 Hz, 2 Hz, 8 Hz, 9.5 Hz, and 25 Hz during cycle 3 ($p < 0.05$). Post-hoc analysis for cyde 1

disclosed significant higher relative EEG activity of young depressed in F and C derivations compared to HO for two delta frequency bins (1.75 Hz and 2.75 Hz) and one theta frequency bin (7.5 Hz) and in addition for the 1.5 Hz and 7.25 Hz frequency bins ($p < 0.05$) for F derivations only. Moreover, higher relative EEG values were observed for MDD compared to HY in F and C derivations in some of the theta frequency bins (7.25-7.5 Hz, $p < 0.01$ for F derivations, $p < 0.05$ for C derivations) during cycle 1. During cycles 2 and 3 there was no significant interaction between groups per derivation after Tukey-Kramer adjustment of p-values.

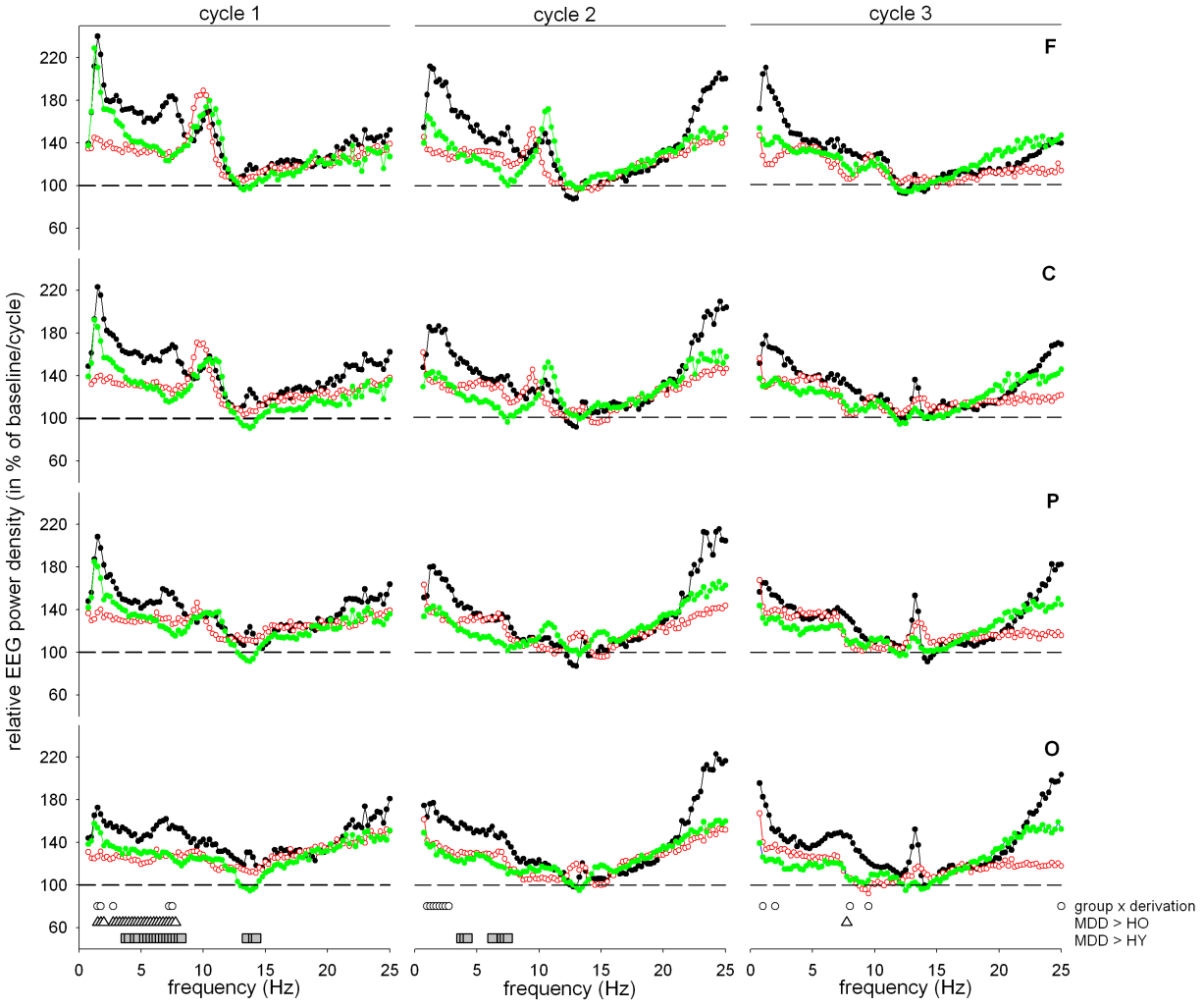

Figure 3.3.2: Relative EEG power spectra per sleep cycle during NREM sleep for the recovery night

*Relative EEG spectra values (EEG power values of the recovery night in % of the baseline values per cycle) are shown between 0.75-25 Hz for F, C, P, and O derivations (collapsed left, central, and right values) for healthy young (HY; green filled circles; n=8), healthy older (HO; red open circles; n=8), and major depressed subjects (MDD; black circles; n=8) for sleep cycles 1-3. Post-hoc comparisons on the significant effect of the factor group (LSMEANS statement p<0.05) as well as significant interaction effects of the main factors group*derivation are indicated at the bottom line (mixed-model 2-way rANOVA p<0.05).*

In order to analyse the time course of the EEG delta activity in detail, percentiles (see methods section) for the frequency range 1-3 Hz (=significant EEG frequency band between groups across baseline and recovery nights; see Fig. 3.3.3) were calculated as a percentage of the mean delta value during the baseline night for NREM sleep cycles 1-3 for F, C, P, and O derivations (see Fig. 3.3.3). A mixed-model 4-way rANOVA disclosed significant effects of the main factors group ($p < 0.01$) with higher delta power activity in young depressed compared to healthy older women ($p < 0.01$; post-hoc analysis based on LSMEANS procedure, Tukey-Kramer adjusted), night ($p < 0.001$) with higher values during recovery night, derivation ($p < 0.001$) with decreasing values along the anterior-posterior axis and cycle ($p < 0.001$) with highest values during sleep cycle 1. Furthermore, significant interaction of the factors group*night ($p < 0.05$), group*derivation ($p < 0.001$), group*cycle ($p < 0.01$), group*night*cycle ($p < 0.001$) and group*night*der*cycle ($p < 0.001$) were observed. Significant within group comparisons for the 4-way interaction group*night*der*cycle are displayed in Figure 3.3.3.

A significant increase in EEG delta power during recovery night was observed for the first NREM sleep cycle within each group for the F and C derivation (MDD and HY $p < 0.001$, HO $p < 0.01$), and for MDD and HY also in the P and O derivation ($p < 0.001$). During NREM sleep cycle 2 there was no significant difference of delta EEG power during recovery and baseline night in any of the derivations in HO. MDDs displayed higher delta activity during recovery night in sleep cycle 2 in each derivation whereas delta power activity in HY was only significantly higher in the F derivations. During NREM sleep cycle 3 no delta EEG activity differences occurred. Post-hoc analysis of the 4-way interaction disclosed significant higher delta power activity of MDD and HY than HO during the first NREM sleep cycle in the recovery night for the F derivation ($p < 0.001$) which was also present during the second NREM sleep cycle between MDD and HO ($p < 0.001$). Neither cycle duration nor NREM or REM duration per sleep cycles 1-3 differed between the three groups during baseline and recovery night ($p > 0.1$, mixed-model 2-way rANOVA; data not shown).

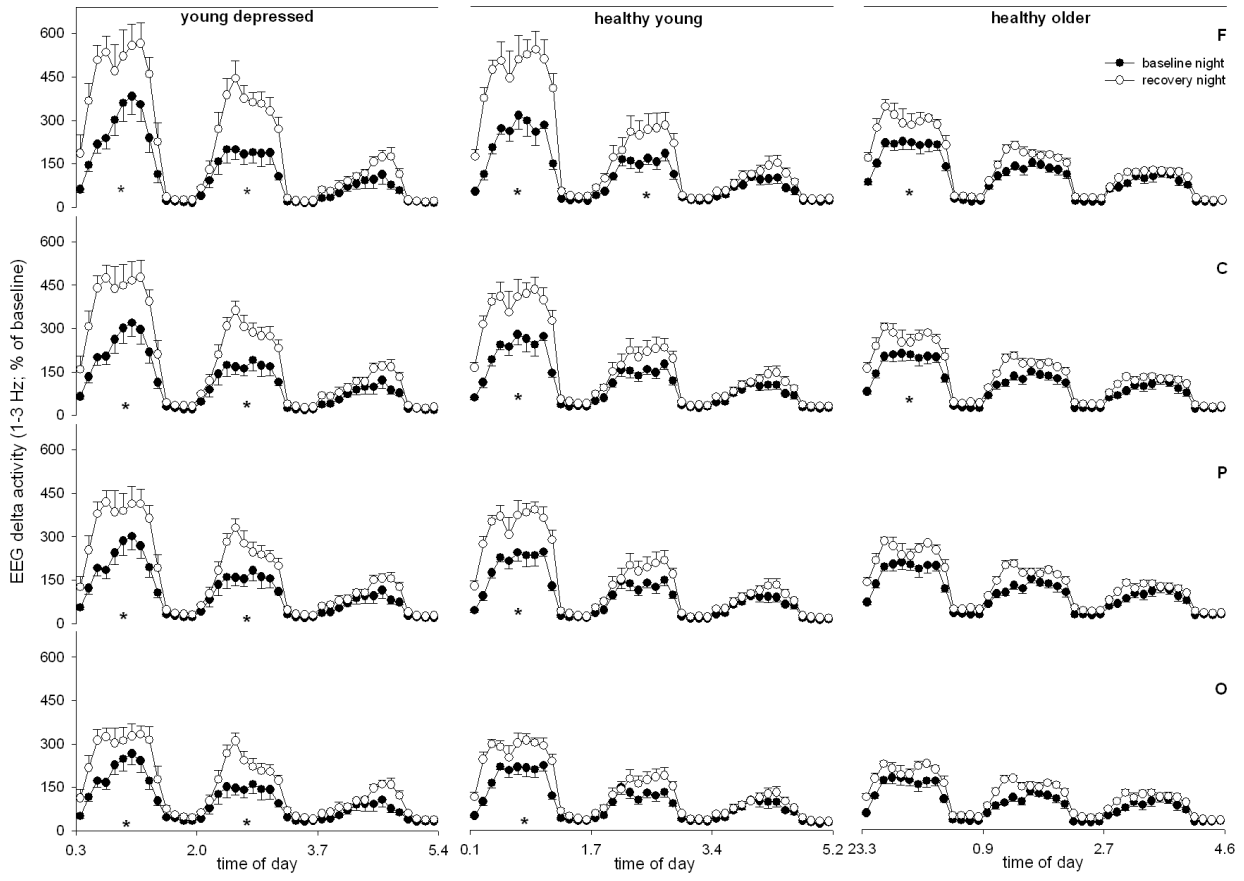

Figure 3.3.3: EEG delta activity per NREM-REM sleep cycles 1-3 during baseline and recovery night

EEG delta activity (1-3 Hz) per sleep cycle 1-3 for collapsed derivations (F, C, P, O) is expressed for each group separately as a percentage of the respective baseline value (young depressed, n = 8; healthy older, n = 8; healthy young, n=8; mean ± SEM). Black filled circles = baseline night, black open circles = recovery night. Asterisks indicate significant post-hoc comparisons of mean values per cycle and derivation between baseline and recovery night within each group (p<0.05; LSMEANS procedure, Tukey-Kramer adjusted).

3.3.3 Ultradian regulation of SWA and delta activity in response to sleep deprivation

Figure 3.3.4 shows the homeostatic response to sleep deprivation as measured by SWA for each group within the first three NREM sleep cycles. Thereby, mean SWA (0.75-4.5 Hz) per sleep cycle during recovery night is expressed in relation to the respective value during the baseline night, commonly known as rebound effect. The sleep deprivation effect becomes quite obvious as SWA during recovery night accounts for more than 100% of the SWA during baseline night. Although the rebound effect in healthy older (HO) women is not as pronounced as in young depressed (MDD) and healthy young (HY) there was no significant difference between groups and neither was a significant effect of the interaction group*cycle observed (p>0.1; mixed-model 3-way rAnova). Post-hoc analysis of the significant interaction group*der within groups disclosed a higher SWA rebound in the F

derivation than in the C, P, and O derivations in MDD and HY ($p < 0.01$ for MDD, $p < 0.05$ for HY; LSMEANS procedure, Tukey-Kramer adjusted). Moreover, MDD showed a significant higher SWA rebound for the F derivations compared to HO ($p < 0.05$).

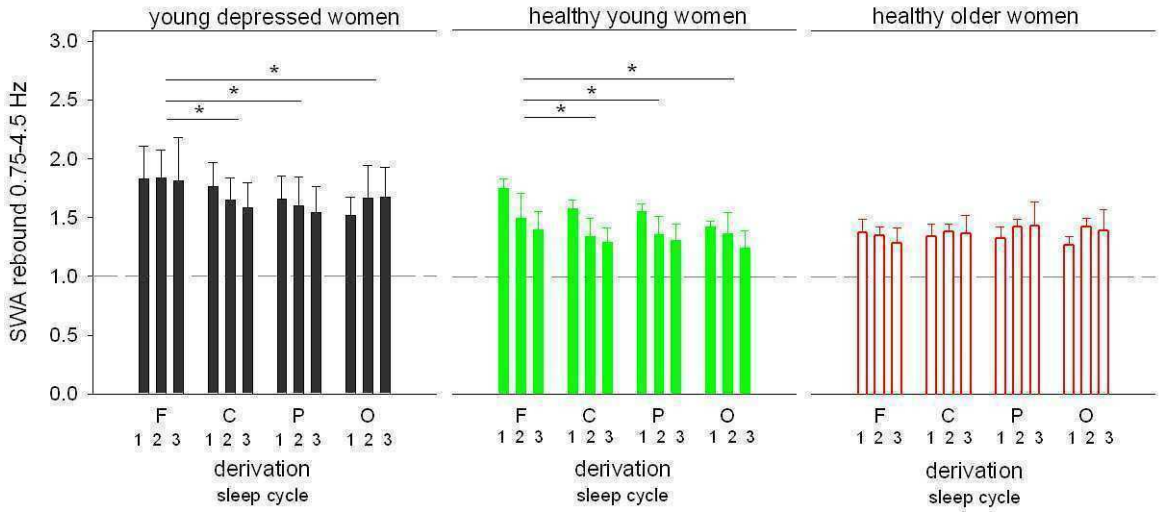

Figure 3.3.4: SWA response to sleep deprivation (SWA rebound)

*The figure illustrates the relative SWA response to sleep deprivation (SWA rebound; SWA during recovery night as a percentage of the SWA during baseline night) for each group for sleep cylces 1-3 for F, C, P, and O derivations (mean ± SEM). Significant post-hoc comparisons of the factors group*derivation within each group are indicated with asterisks ($p < 0.05$; LSMEANS statement with Tukey-Kramer adjusted p values).*

Delta ratios (delta EEG activity during NREM sleep cycle 1 in relation to the respective value during sleep cycle 2) of HY were significantly higher than of MDD and HO during recovery night ($p < 0.05$; mixed-model 2-way rANOVA; data not shown) which is a reflection of the different time-dependent decrease of SWA in HY compared to the other groups with visibly (but not statistical significant) less SWA during cycle 2 than in the first sleep cycle (see Fig. 3.3.4).

Figure 3.3.5 displays mean EEG delta power (0.75-4.5 Hz) during NREM sleep along the antero-posterior axis for the baseline and recovery nights for each group. Mixed-model 3-way rAnova disclosed significant effects of the main factors group, derivation, and night with higher mean delta values in young depressed (MDD) compared to healthy older (HO) women ($p < 0.05$), highest mean values in F derivations ($p < 0.001$), and with higher mean EEG delta power activity during recovery night compared to baseline night ($p < 0.001$) respectively. There were significant interactions of the factors group*der ($p < 0.001$), group*night ($p < 0.01$) and group*der*night ($p < 0.001$). Post-hoc analysis of the 3-way interaction showed significant higher EEG delta activity in the F derivation of MDD than HO during baseline night ($p < 0.05$) and recovery night ($p < 0.001$) as well as higher values in the F derivation of MDD than HY during recovery night ($p < 0.05$).

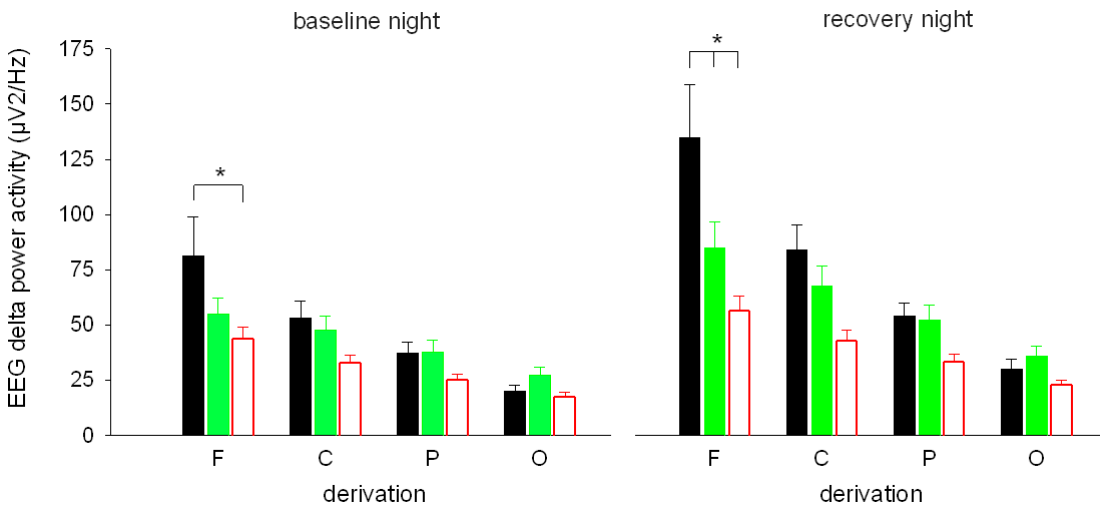

Figure 3.3.5: Mean EEG delta power activity along the antero-posterior axis during baseline and recovery night

Mean EEG delta power activity ±SEM during NREM sleep is displayed for lateral and central collapsed F, C, P, and O derivations for each group (young depressed women = black bars; healthy young women = green bars; healthy older women = red open bars). Significant post-hoc comparisons between groups and derivation for the baseline and the recovery night are indicated with asteriks ($p < 0.05$; post-hoc analysis based on LSMEANS statement with Tukey-Kramer adjustment of p values).

Moreover, post-hoc analysis on the 3-way interaction within each group showed a significant frontal predominance of mean EEG delta activity in MDDs in F derivations compared to P, and O derivations during baseline night and of F compared to C, P, and O derivations during recovery night ($p < 0.001$). In contrast, HY showed significant differences in mean EEG delta activity only between F and O derivations during recovery night with higher values in the F derivation ($p < 0.001$). In HO no frontal predominance was observed ($p > 0.05$) during baseline and recovery night (Fig. 3.3.5). A colour coded topographic map for the baseline and the recovery night with mean delta values for each group was added in Figure 3.3.6 in order to better emphasize the hyperfrontality of SWA in young depressed compared to the two healthy groups.

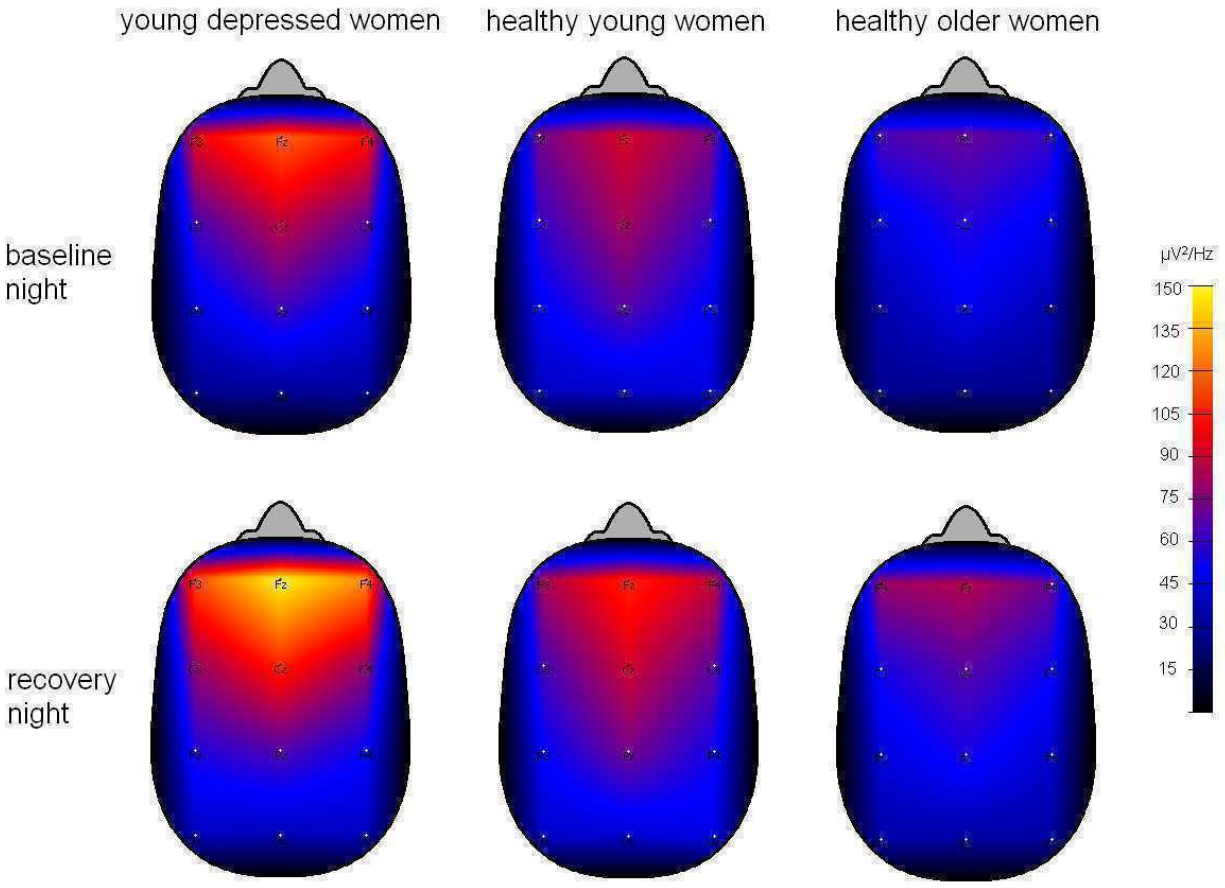

Figure 3.3.6: Topographic map of mean SWA during NREM sleep

Mean SWA (0.5-4.75 Hz) during NREM sleep was calculated for each subject before averaging across groups for 12 electrodes (Fz, F3, F4, Cz, C3, C4, Pz, P3, P4, Oz, O1, O2). Slow-wave activity values are colour coded as indexed by the right-hand scale whereby blue represents low values and yellow very high values. Young depressed women n=9; healthy young women n=8; healthy older women n=8.

3.3.4 Decay of slow-wave activity during baseline and recovery nights

The decay of the SWA for each group during baseline and recovery night was analysed by fitting a nonlinear regression function (see methods section) to the individual mean spectra values centred at the middle of each sleep cycle of each participant in the relative EEG delta range (1-3 Hz; percentage of baseline night). Figure 3.3.7 shows the fitted exponential decay function for each of the three groups during the baseline and recovery nights for lateral collapsed F derivations. The respective estimated parameters of the decay functions are displayed in Table 3.2.

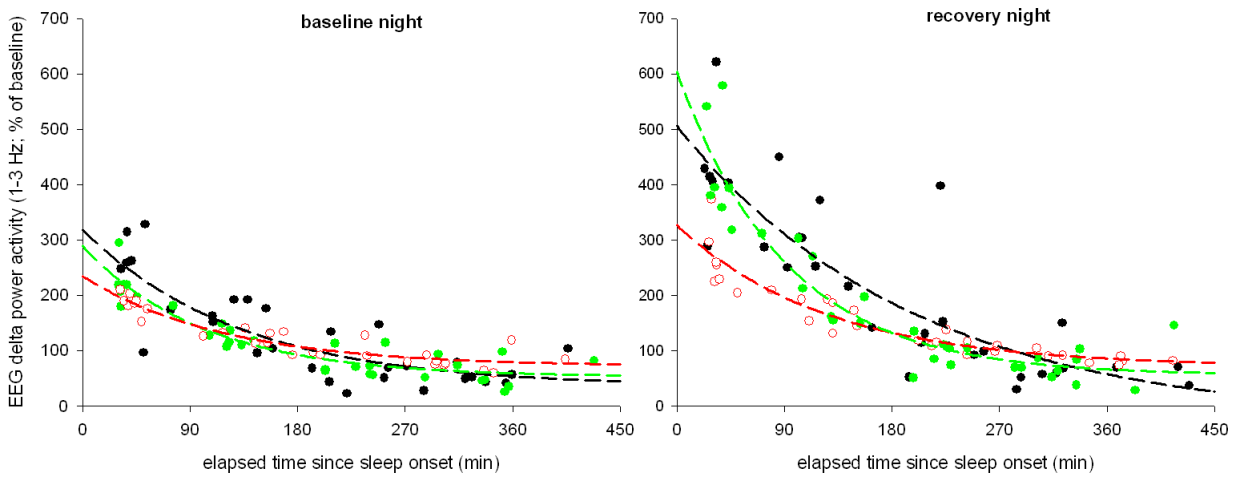

Figure 3.3.7: Fitted exponential decay of relative delta power activity during baseline and recovery night

Fitted exponential decay function [$delta_t = delta_\infty + delta_0 * e(-rt)$] to relative EEG delta power (1-3 Hz; percentage of baseline night) NREM sleep across all NREM sleep episodes for lateral collapsed F derivations during baseline (left hand panel) and recovery night (right hand panel). Major depressed women n = 9, black circles; healthy young women n = 8, green circles; healthy older women n = 8, red open circles.

The difference of the decay rates between groups was not significant since the the mean decay rates of each group reached the 95% confidence interval of the other two groups during baseline night and during recovery night. Furthermore, mean values of the baseline slopes overlapped with the 95% confidence interval of the recovery nights within the groups. Interesting, although not significant, young depressed and healthy older women exhibited a shallower decline of SWA during the recovery night which contrasts the steeper slope of healthy young after sleep deprivation.

Table 3.2: Estimated parameters for the nonlinear regression analysis of the decay of relative EEG delta power activity during baseline and recovery nights

parameter	young depressed women		healthy young women		healthy older women	
	baseline night	recovery night	baseline night	recovery night	baseline night	recovery night
Decay rate, /min	0.0076 ±0.0033	0.005 ±0.0024	0.0098 ±0.0023	0.0109 ±0.0022	0.0084 ±0.0022	0.0079 ±0.0019
95% CI	0.000793-0.0144	0.000069-0.01	0.00525-0.0145	0.006-0.0155	0.00402-0.0128	0.00402-0.0119
R	0.84	0.86	0.93	0.94	0.94	0.94

The estimated parameters of the nonlinear regression analysis for the decay of EEG delta power during baseline and recovery nights for all three groups are indicated for the collapsed F derivations during all NREM sleep episodes (mean of all cycles ± SEM). 95% CI = 95% confidence interval.

3.3.5 Time course of circadian variables

Salivary melatonin, as a reliable variable to measure endogenous circadian rhythmicity, was sampled every 30 minutes over the entire 40 hours protocol. Samples were subsequently collapsed into 2.5 hour bins per subject before averaging across groups (see Fig. 3.3.8). Mixed-model 2-way rAnova disclosed significant effects of the main factors group ($p < 0.05$) and session ($p < 0.001$) as well as of their interaction ($p < 0.001$). Post-hoc analysis on the group effect showed thereby only a tendency to significant higher mean melatonin secretion in healthy young compared to healthy older women ($p = 0.057$) during the 40 hour protocd. Moreover, healthy young had significantly higher nocturnal melatonin levels compared to young depressed in session 8 ($p < 0.001$) and than healthy older during sessions 7 and 8 ($p < 0.05$ and 0.001 respectively).

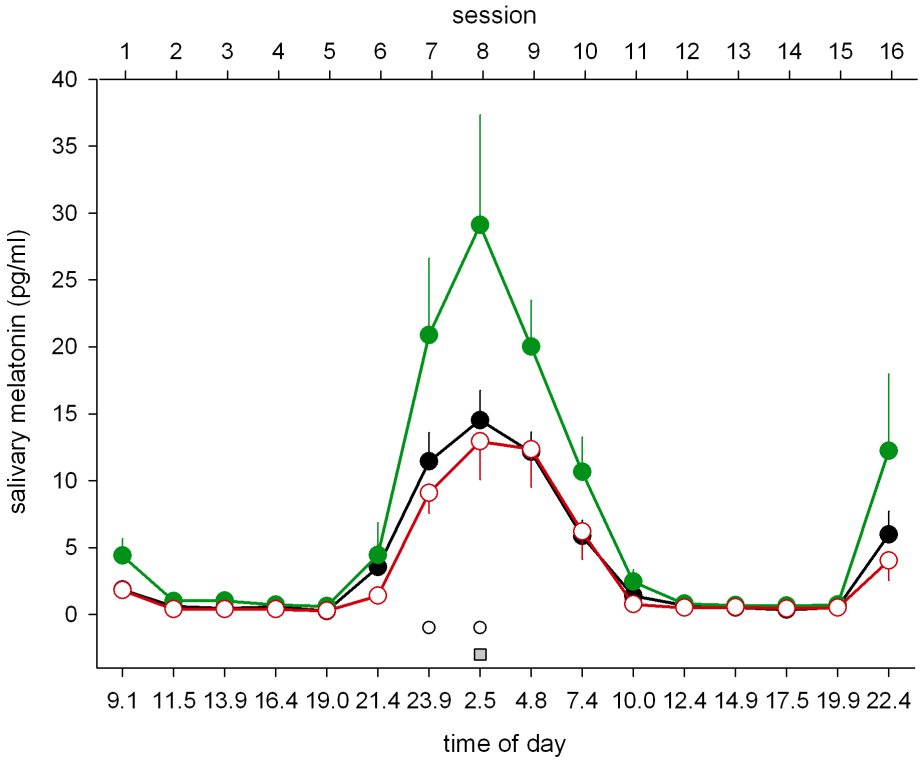

Figure 3.3.8: Mean salivary melatonin levels during the 40-h sustained wakefulness protocol

*The figure displays mean salivary melatonin values (± SEM) for healthy young (HY, green filled circles; n=8), young depressed (MDD, black filled circles; n=9), and healthy older (HO, red open circles; n=8) volunteers. Significant post-hoc comparisons of the interaction of the factors group*session are indicated at the bottom line (open circle = HY > HO, filled square = HY > MDD; p at least < 0.05; post-hoc analysis based on LSMEANS statement with Tukey-Kramer adjustment of p values).*

Subjective sleepiness ratings were derived from the Karolinska Sleepiness Scale (KSS). They provide useful supplemental information to polysomnographic measurements during sleep. The time course of mean subjective sleepiness per group as illustrated in Figure 3.3.9 (top panel) is based on half-hourly ratings which were collapsed into 2.5-h bins. Mixed-model

2-way rAnova disclosed only significance for the factor session ($p < 0.001$) but not for the factor group or the interation between these two factors.

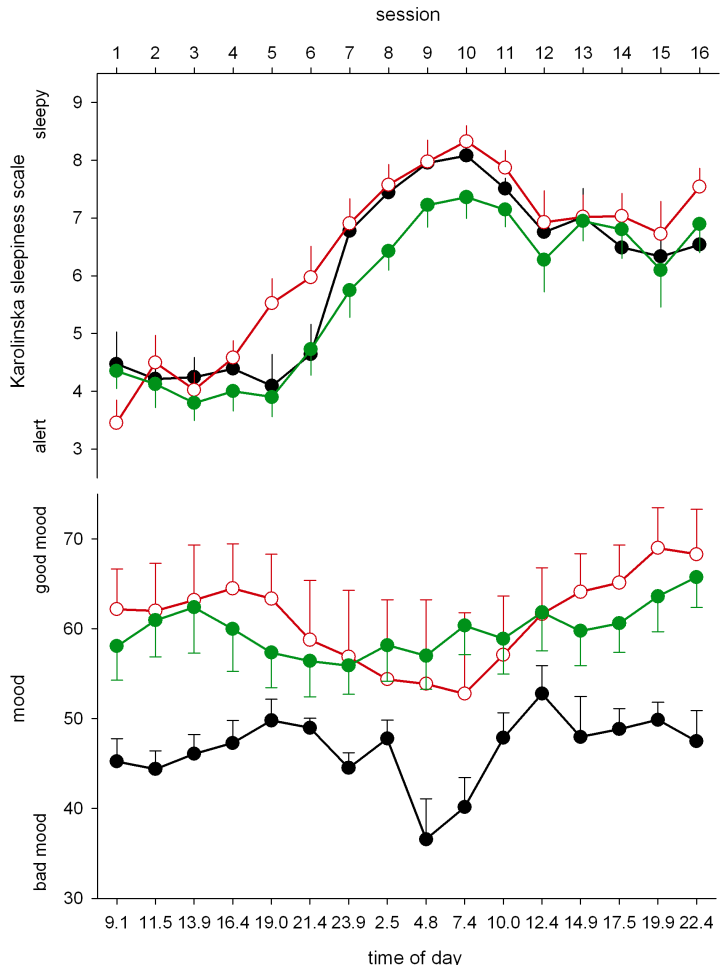

Figure 3.3.9: Time course of subjective sleepiness and mood during 40-h of sustained wakefulness

Mean subjective sleepiness (top panel) and mean mood (bottom panel) ratings are shown for each group over the 40-h period between baseline and recovery night whereby half-hourly given subjective ratings were binned in 2.5-h segments (means ± SEM). Subjective sleepiness ratings are derived from the Karolinska Sleepiness Scale whereas subjective mood ratings are based on the visual analogue scale. HY = healthy young (green filled circles; n=8), HO = healhy older (red open circles; n=8), MDD = young depressed (black filled circles; n=9). Groups did not differ significantly with reference to sleepiness (mixed-model 2-way rAnova). In contrast, MDD exhibited significantly lower mood ratings than HY and HO ($p<0.05$; post-hoc analysis based on LSMEANS statement, Tukey-Kramer adjusted) (mixed-model 2-way rAnova).

The time course of subjective mood ratings during sustained wakefulness was significant ($p < 0.001$; panel at the bottom of Figure 3.3.9). Furthermore, the mood of young depressed was signficantly worse compared to the two healthy groups ($p < 0.05$). Interestingly, no significant mood improvement occured in MDD during sleep deprivation with respect to both, the value before baseline night (data not shown) or the start value during the sustained wakefulness protocol ($p > 0.1$). The interaction of the factors group*session was not significant.

3.4 Discussion

We have investigated the impact of high sleep pressure induced by 40 hours of sustained wakefulness on sleep architecture and sleep EEG spectra in young moderately unipolar depressed women (MDD) and healthy young (HY) as well as in older controls (HO). All three groups showed a typical increase of slow-wave activity (SWA) during the first part of the recovery night as a response to increased homeostatic sleep pressure [81]. Young depressed reacted with an even stronger absolute SWA response in the delta frequency range compared to healthy young and healthy older women and particularly in frontal brain regions. Furthermore, also relative EEG spectra showed higher homeostatic response to sleep deprivation in depression compared to healthy young and older, particularly in the first sleep cycle but also in the second sleep cycle when compared to healthy young. In both young groups, depressed and healthy, the low frequency EEG response to sleep deprivation was accompanied by a hyperfrontality which not only confirms the fact that frontal brain areas are most susceptible to sleep deprivation [82] but also extends previous findings on this topographical feature of NREM sleep homeostasis in middle-aged depressed outpatients [267] to a younger moderately depressed cohort. In contrast, healthy older women exhibited not only attenuated SWA values compared to the other two groups but also an absence of the frontal predominance of mean SWA during the recovery night along the antero-posterior axis which confirms previous findings on age-related changes in homeostatic sleep regulation after sleep deprivation [278, 100].

Homeostatic response to sleep deprivation

Our data show no deficiency in the homeostatic regulation of sleep (process S) in young moderately depressed women which contrasts with the predictions of the S-deficiency hypothesis [113] that there is a reduced SWA response during sleep in depression after sleep deprivation due to an impaired buildup of homeostatic sleep pressure during wakefulness. However, it has to be noted in this context that the S-deficiency hypothesis was developed on the basis of two important clinical findings: first, the common occurrence of sleep disturbances in depression and, second, the clinical effect of sleep deprivation to improve mood in depressed patients. Our group of depressed women had none of the reported common sleep disturbances such as reduced sleep time, increased REM sleep and shortened REM sleep latency for example as indexed by the quite common sleep stage durations presented in this study. Moreover, we could not observe a mood improvement in our depressed subjects during the 40-h sustained wakefulness protocol. Hence, our depressed subjects presumably represent a subgroup in depression where the S-deficiency hypothesis may not apply. Altogether, our findings add a caveat to the S-deficiency hypothesis rather than refute it. A homeostatic deficiency in sleep regulation may be state dependent. As such it may be related to the presence of sleep disturbances associated with depression such as insomnia for example rather than to depression per se. The limitation of this conclusion is of course

its inverse argumentation which would need validation by a 40-h sleep deprivation study of age-matched depressed women with sleep disturbances under the same stringent conditions of a constant routine protocol as presented in this study.

Our results on homeostatic SWA response to sleep deprivation in young depressed women corroborate previous findings in depressed female outpatients aged 18-40 years where an enhanced SWA response to a relatively small homeostatic sleep pressure after a 3-hour sleep delay was found compared to healthy controls [266, 279]. As our study included outpatients of approximately the same age range and due to a lack of studies on this subject, it still remains unclear whether younger or older depressed women would exhibit a similar EEG delta response to sleep deprivation compared to healthy age-matched controls. Furthermore, the stronger frontal SWA response during the recovery night in our depressed group after sleep deprivation shows not only that homeostatic sleep regulation is different from that in healthy aging but also points toward higher homeostatic sleep pressure in moderately depressed women compared to age-matched healthy controls. The latter was not caused by less sleep prior to sustained wakefulness as total sleep time during the baseline night was not different between the groups. Our recent findings of higher delta and theta EEG activity during sustained wakefulness, as a marker of sleep homeostasis and sleep propensity [280, 281], in the same cohort of depressed subjects compared to healthy young further evidence this conclusion (unpublished data).

Ultradian modulation of EEG slow-wave activity during NREM sleep

The dynamics of SWA with reference to its exponential decline during the night did not show significant different time constants between the three groups and the high R2 values confirmed a good quality of fit of the decay functions. Although not significant it is thereby noteworthy that the decay rate during recovery night compared to baseline night showed a decreasing trend in young depressed and healthy older women while in healthy young there was an opposite pattern observed. The decreasing time constant in MDD and HO may be explained by a different ultradian modulation of NREM delta activity after sleep deprivation compared to HY as indicated by a rather stable slow-wave activity rebound during the first 3 sleep cycles in MDD and HO compared to a consecutive declining trend over the sleep cycles in HY during the recovery night. Additionally, and even more pronounced, differences in ultradian delta sleep modulation became obvious by inspection of the delta sleep ratios (the ratio of the NREM delta activity during sleep cycle 1 and sleep cycle 2 in the frequency range 0.75-4.5 Hz) where young depressed and older women exhibited significant lower values after sleep deprivation compared to healthy controls. The occurrence of lower delta sleep ratios thereby has first been described by Kupfer and colleagues and they found also a positive correlation of the level of the delta sleep ratio and the duration of the remission times in depression [202]. Other studies showed an age-dependency of delta sleep ratios in depression as reduced values were found in adolescent depressed women compared to healthy controls but not in depressed women aged 18-40 years [282**?**]. Furthermore, lower delta sleep ratios

were found in older depressed women independent of their remission state [283]. In contrast to these other study findings, where lower mean SWA values were found during the first NREM sleep cycle compared to the second, low delta sleep ratios in our group of women with major depression after sleep deprivation came about a higher temporal preponderance of SWA during sleep cycle 2 compared to healthy controls as visible in the time course of the EEG delta activity percentiles per NREM sleep cycle. With regard to the two-process model of sleep regulation, this different temporal pattern of NREM delta activity is under circadian control such as are other temporal related aspects of sleep regulation [53, 72, 55]. Hence, lower delta sleep ratios may point towards circadian alterations in sleep regulation which at least for our older group would be supported by previous suggestions of a reduced ability of the hypothalamic circadian master clock to synchronise endogenous rhythms [284].

Melatonin secretion and subjective sleepiness

We observed lower mean melatonin levels in depressed subjects and older compared to healthy controls. These findings coincide with other reports of low melatonin levels for both groups [285, 286, 287, 288]. Reduced melatonin levels may indicate a reduced signal of circadian components responsible for the promotion of wakefulness during the biological day according to the two-process model of sleep regulation. There are many reports of circadian disturbances in depression [216, 186], but to date no constant routine studies have been performed to elucidate unmasked circadian aspects of sleep-wake regulation in major depression. In healthy older women there is some evidence for a weaker circadian signal in sleep-wake regulation as it has been shown under constant routine conditions that more sleep occurs in older healthy compared to young healthy particularly in the so-called forbidden zone for sleep [289] which refers to the period of wakefulness immediately prior to the increase of sleep propensity in healthy young [278].

We observed no significant differences in subjective sleepiness between young depressed and healthy young and older women during the 40 hours of sustained wakefulness. However, visual inspection of the time course of subjective sleepiness showed a marked increase of sleepiness in healthy older women in the early evening after approximately 10 hours of wakefulness compared to the other two groups and in young depressed after approximately 14 hours compared to healthy young. Both increases may reflect a lower circadian arousal for wakefulness.

Conclusions

Our study results show that homeostatic sleep regulation as well as sleep architecture in young moderately unipolar depressed women is not equal to premature aging as noted by some authors [195, 196]. Moreover, our findings show that young moderately unipolar depressed women live on higher homeostatic sleep pressure and we hypothesise that a reduced

circadian arousal signal during wakefulness may contribute to this. However, a major limitation to this conclusion is that alterations of the circadian sleep-wake regulation may not adequately studied by our current study setting where sleep occurred at habitual sleep time. Hence, a protocol should be used where sleep occurs a different circadian phases with preferably similar prior amount of wakefulness in order to avoid confounded results by different levels of homeostatic sleep pressure.

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4 Young women with major depression live on higher homeostatic sleep pressure than healthy controls

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Abstract

There is mounting evidence for the involvement of the sleep-wake cycle and the circadian system in the pathogenesis of major depression. However, only a few studies so far focussed on sleep and circadian investigations under distinct unmasking experimental conditions. Thus, it remains unclear whether homeostatic sleep pressure or circadian rhythms or both are altered in depression. Here, we aimed at quantifying homeostatic and circadian sleep-wake regulatory mechanisms in young women suffering from major depressive disorder and healthy controls during a multiple nap paradigm under constant routine conditions.

After an 8-h baseline night nine depressed women, eight healthy young and eight healthy older women underwent a 40-h multiple nap protocol (10 short sleep-wake cycles) followed by a 8-h recovery night under constant environmental conditions. Polysomnographic recordings were carried out continuously and subjective sleepiness was assessed. In order to measure endogenous circadian rhythm signal output, salivary melatonin samples were collected during scheduled wakefulness and the circadian modulation of sleep spindles has been analysed with reference to the timing of the melatonin secretion. Sleep parameters as well as NREM sleep EEG spectra for collapsed left, central and right frontal, central, parietal, and occipital derivations for the night and nap sleep episodes in the frequency range 0.75-25 Hz were analysed.

Young depressed women showed higher frontal EEG delta activity, as a marker of homeostatic sleep pressure, compared to healthy young and older women across both night sleep episodes together with significantly higher subjective sleepiness levels. Higher delta sleep EEG activity in naps during the biological day were observed in young depressed along with a reduced nighttime melatonin secretion as observed also in healthy older compared to healthy young volunteers. The circadian modulation of sleep spindles between the biological night and day was virtually absent in healthy older and partially impaired in young depressed women.

Our data provide strong evidence for higher homeostatic sleep pressure in young moderately depressed women along with some indications for an impairment of the strength of the endogenous circadian output signal involved in sleep-wake regulation. The latter may be conducive for an overexpression of sleep-wake homeostatic processes. This finding may have important repercussions on the treatment of the illness in this endophenotype of depression as such that a decrease of homeostatic sleep drive by selective suppression of EEG slow-wave activity could promote acute mood improvement.

4.1 Introduction

Major depression today is one of the main causes for disease affecting at least 121 millions of people worldwide with the highest rates of disabilites [138]. Lifetime prevalence for major depression in the US accounts for 16.2% with a more than 1.5 higher risk for women than men [290]. Hormonal fluctuations associated with the female reproductive lifecycle on the levels of neurotransmitters such as serotonin and noradrenaline are believed to be involved in the pathophysiology of depression [147, 148]. Vulnerability to depression has been shown to be partly related to genetic and environmental factors such as stress, emotional trauma, and viral infections or their interaction with genetic predisposition [152, 157, 257, 258]. Moreover, there is compelling evidence for an impairment of structural and functional brain plasticity associated with the pathophysiology of major depression [183, 175, 176, 149]. However, despite considerable research effort during serveral decades, the etiology and pathophysiology of major depression as a rather idiopathic illness remains fragmented [149, 150, 151].

More than 80% of patients with major depression also suffer from insomnia, while 15-35% show signs of hypersomnia [184, 185, 206]. EEG studies in major depression usually report abnormalities in sleep architecture including prolonged sleep latency, shortened REM sleep latency and increased REM sleep, decreased slow-wave sleep and EEG slow-wave activity, and a higher rate of wake up incidents particularly towards the end of the night sleep episode [191, 185, 192, 193, 187, 194, 259, 199]. Interestingly, most of these changes in sleep architecture are also typically observed in healthy aging without any indication of a depressive symptomatology [195]. However, in contrast to the literature on age-related sleep alterations [291, 292, 293], the findings on depression-related sleep disturbances are rather inconsistent [205**?** , 206, 161]. Major limits in highlighting common markers of sleep-wake disruptions and circadian rhythm abnormalities in depression encompass the strong evidence for the existence of different endophenotypes in depression [160, 161].

The sleep-wake cycle as most other behavioral and physiological processes such as melatonin secretion, alertness, and many cognitive functions follow a circadian rhythmicity generated by a central pacemaker in the suprachiasmatic nuclei (SCN) located in the anterior hypothalamus [56, 55]. According to the two-process model of sleep regulation, sleep and wakefulness is coordinated by the interaction of a circadian process C and a sleep-wake dependent homeostatic process S [53]. Thereby, the timing, duration and consolidation of sleep is mainly controlled by process C whereas process S reflects the increasing need for sleep during wakefulness and dissipates during NREM sleep [39]. The two processes C and S interact in an opponent manner such that the circadian process promotes wakefulness during the biological day and thereby counteracting the continuous build-up of sleep pressure (process S) with increasing time awake [51, 55]. Interestingly, subjective mood levels also depend to a substantial degree on the contribution of the homeostatic and circadian processes in healthy study volunteers [294, 295] and in women suffering from major depression [227]. Hence, endogenous circadian rhythm alterations may not only be involved in the development of sleep

disturbances in major depression but also in the pathogenesis of the disease[216, 296]. In fact, several circadian disturbances have been reported in major depression such as elevated nocturnal core body temperature, increased cortisol secretion, decreased plasma melatonin levels and phase-advances (earlier timing) of the circadian rhythm of all of these mentioned variables [215, 216, 296]. Despite the fact that circadian alterations in depression have not been confirmed by all studies [186, 189], several hypotheses have been developed with reference to circadian, ultradian or homeostatic abnormalities in depression such as the so-called S-deficiency hypothesis [113], the phase-advance hypothesis [108], the low melatonin hypothesis [297], and the acetylcholine-monoamine imbalance hypothesis [264].

The relative contribution of circadian and homeostatic processes to sleep regulation can only be assessed by applying different sleep-wake manipulation schemes under controlled laboratory conditions in order to dissect the two processes and to unmask endogenous circadian rhythms from external Zeitgebers such as light, temperature, food intake, and social interactions [224]. Thus, here we aimed at investigating homeostatic and circadian sleep regulation under low sleep pressure conditions by applying short sleep-wake cycles (naps) distributed over a 40-h period allowing for sleep episodes to occur at different circadian phases. Age and sex matched healthy young and sex matched healthy older control volunteers were included in the study. The rationale behind including healthy older volunteers in our study was to determine whether reported similarities in sleep disturbances in major depression and in healthy older underly common changes in circadian and homeostatic sleep-wake regulation. To our knowledge, a nap protocol under stringently controlled laboratory conditions (i.e. constant routine conditions) has never been applied in major depression before. Our main prediction was that young depressed women in contrast to young healthy women show a reduced circadian wake-promoting signal in the early evening as indexed by more sleep during nap episodes scheduled during this time of day as it has been shown in healthy aging [278]. Furthermore, we expected a different phase position (either advanced or delayed) of the circadian rhythm in salivary melatonin in young depressed compared to both healthy control groups.

4.2 Methods

4.2.1 Study participants

Female study participants were recruited via advertisements at different Universities in the region of Basel, Switzerland and via selected online portals. Eight healthy young (HY; 20-31y, mean = 25.4y +/- SD 3.8y), eight healthy older (HO; 57-74y, mean = 64.4y +/- SD 5.4y), and nine young women with major depressive disorder (MDD; 19-32y, mean = 22.8y +/- SD 3.9y) were considered in the study. A two sided t-test disclosed no significant age differences between young depressed and healthy young volunteers.

All participants underwent a defined screening procedure encompassing questionnaires to screen physical health, drug consumption, and sleep quality as well as a medical examination to assess somatic state. Additionally, young depressed volunteers filled in a self-reported depression rating questionnaire (Beck Depression Inventory (BDI)[268] whereby only participants with a score >12 were considered for a subsequent clinical screening. The mean BDI value of considered study participants was 20.3 ± SD 8.5. To assess the presence of a major depressive disorder a structured clinical interview for DSM-IV Axis I (SCID-I) according to the DMS-IV_R (Diagnostic and Statistical Manual of the American Psychiatric Association, 1994) was carried out with the respective MDD volunteers (mean SCID-I value = 5.9 ± 1.4). The considered study participants thereby had no atypical symptoms and no psychiatric comorbidity according to DSM-IV_R. Healthy young and healthy older participants had no sleep disturbances as assessed by the Pittsburgh Sleep Quality Index (PSQI)[13]) (PSQI value ≤5) whereas for MDD participants a score ≤8 was allowed (i.e. only mild forms of sleep disturbances) (MDD = 6.9 ± 1.4, HY = 2 ± 1.7, HO = 3.9 ± 1.6; mean +/- SD). Thereby, the PSQI values of healthy young women was significantly lower compared to the other two groups ($p<0.05$), and the young depressed women had significant higher values compared to healthy older ($p <0.001$). Additionally, all participants followed an adaptation night in the chronobiology laboratory in order to evaluate sleep quality by polysomnographic recordings and to exclude volunteers with a sleep efficiency of less than 80%, periodic leg movements of more than 10 per hour, and a apnea-hypopnoea index >10. Medications other than oral contraceptives were not allowed for all volunteers. All study participants were drug-free (verified by urinary toxicologic analysis), nonsmokers, and had no shift work or flights over more than 3 time zones during the last 3 months before the study began. Only intermediate chronotypes as assessed by the diurnal type scale [11] were considered (MDD = 16.8 ± 1.7, HY = 15.6 ± 3.8, HO = 17.5 +/- 4; mean ± SD; a t-test yielded no significant differences between the groups). All volunteers participated in the laboratory part of the study during the follicular phase of their menstrual cycle (days 1-5 after mensis onset). All study participants gave their signed informed consent for the participation in the study. The study procedures as well as all questionnaires and the consent form were approved by the local Ethics Committee of Basel (EKBB), Switzerland. All procedures conformed to the Declaration of Helsinki.

4.2.2 Study protocol

The study comprised a 7-day ambulatory part at home followed by a laboratory part (3.5 days). During the ambulatory part volunteers were asked to restrict their caffeine intake to only one beverage per day, to drink not more than 5 alcoholic drinks during the entire week, and to abstain from heavy physical exercise. Furthermore, they were asked to keep a regular sleep-wake schedule during the ambulatory study part prior to admission to the laboratory. Compliance was verified by sleep logs and ambulatory activity measurements (wrist activity monitor, Cambridge Neurotechnology Ltd®, UK). The timing of the sleep-wake schedule

during the protocols was adjusted to individual habitual bedtimes calculated by centering the approximate 8-hour sleep episodes during the baseline week at the individual midpoint of sleep of each participant. Habitual bedtimes did not significantly differ between the three groups (young depressed 11:53 PM ± SD 58 minutes, healthy young 11.37 PM ± SD 81 minutes, healthy older women 11:24 PM ± SD 52 minutes). The protocol comprised a habituation night followed by a baseline night in the chronobiology laboratory. After the baseline night the participants followed a 40-hour multiple nap protocol with 10 alternating sleep-wake cycles of 75/150 minutes duration which was followed by a 8-hour recovery night at habitual bedtimes (Fig. 4.2.1).

While in the laboratory, participants received no external time cues. Polysomnographic recordings started in the afternoon after the habituation night. During the entire protocol participants remained under constant conditions such as dim-light < 8 lux during wakefulness and 0 lux during sleep episodes, semirecumbent posture position in bed during scheduled wakefulness, regular isocaloric meals, and constant room temperature. A daily heparin injection was given to the older volunteers in order to prevent venous thrombosis (Fragmin®, 0.2 mL, 2500 IE/UI, Pfizer AG, Switzerland). The severity of the depressive episode of young volunteers with major depression was assessed regularly (see Fig. 4.2.1) by an independent psychologist on the basis of the Montgomery-Asperg Depression Rating Scale (MADRS)[269] and the Hamilton Rating Scale for Depression (HAMD-17) [270].

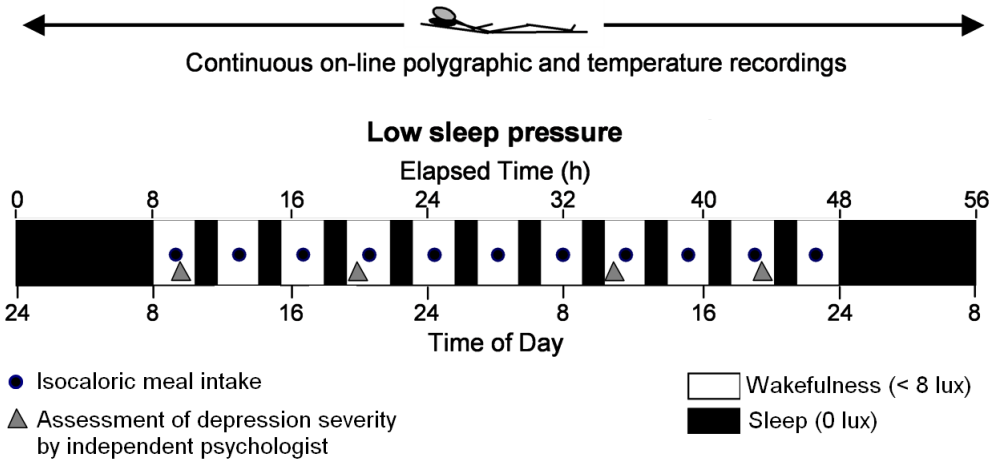

Figure 4.2.1: Schematic illustration of the short sleep-wake cycle (nap) protocol

The illustration shows the timing of sleep and wakefulness, food intake, and the psychological assessments of depression severity in young women with major depression in the course of the nap protocol. As a consequence of the alternating sleep/wake cycles of 75 and 150 minutes respectively across a time span of 40 hours, sleep pressure levels remain constantly low. The protocol required bedrest conditions and comprised a baseline night and a recovery night. The timing of the sleep and wake episodes, body posture, light levels, and room temperature were stringently controlled (i.e. constant routine conditions).

4.2.3 Polysomnographic recordings and analysis

Polysomnographic recordings (Vitaport-3 digital recorder TEMEC Instruments BV, Kerkrade, the Netherlands) during sleep comprised twelve EEG derivations (F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, O1, O2, Oz referenced against linked mastoids), two electrooculograms, one submental electromyogram, and one electrocardiogram. All EEG signals were filtered at 30 Hz (fourth-order Bessel-type antialiasing low-pass filter, total 24 dB/Oct), and a time constant of 1.0 second was used prior to online digitization (range 610 µV, 12 bit AD converter, 0.15 µV/bit; storage sampling rate at 128 Hz). Sleep episodes were visually scored on a 20-second epoch basis according to the standard criteria of Rechtschaffen and Kales [29]. EEGs were subjected to spectral analysis using a fast Fourier transform (FFT) with 10% cosine 4-second windows resulting in a 0.25 Hz resolution. Sleep stages (1-4), REM sleep, and movement time (MT) were expressed as percentage of total sleep time (TST) during the respective night for all participants. TST and sleep latencies to stage 1 (SL1), stage 2 (SL2), and REM (RL) were indicated in minutes. Sleep efficiency (SE) was expressed as percentage of TST of the total time between lights off and lights on (TIB). Wakefulness after lights off (WALO; % of TIB) and wakefulness after sleep onset (WASO; % of TST) were also calculated. NREM sleep was defined as stages 2 to 4 (% of TST). Sleep parameters during the 10 naps were similarly calculated on the basis of the TST during all 10 naps. The time course of different sleep stages during the 10 nap episodes was based on visual sleep scoring of selected sleep stages and is illustrated in Figure 4.3.1.

EEG spectra during the baseline and recovery nights were calculated for the first 7 hours of NREM sleep in the frequency range from 0.75 to 25 Hz for all 12 EEG derivations. A mixed-model 4-way rAnova with the factors group (young depressed vs healthy young vs healthy older women), night (baseline vs recovery), derivation site (frontal vs central vs parietal vs occipital), and hemisphere (left vs right) disclosed a significant lower left hemisphere EEG values for some of the frequency bins of the delta, theta, and beta range (0.75 Hz, 4.25-4.5 Hz, 4.75-6.5 Hz, 17.75-18.75 Hz, 20.75-21.25 Hz, 22-22.25 Hz, 23-23.5 Hz) as well as a significant interaction of the factors group*hemisphere*derivation site in three frequency bins of the sigma range (13.75 Hz, 14.5-14.75 Hz) ($p < 0.05$). Post-hoc analysis yielded no significant differences between groups and hemisphere per derivation site (p at least $>$ 0.05; post-hoc analysis based on LSMEANS statement; Tukey-Kramer adjusted). No other significancies were observed (p at least > 0.05). Hence, spectral values for side and midline derivations were collapsed afterwards along the anterior-posterior axis resulting in one value for each of the F, C, P, and O sites. Baseline and recovery night values for each protocol were graphically illustrated as percentage of the respective values of the HY (100%) whereby the statistical analysis was based on absolute spectra values.

NREM-REM sleep cycles were defined according to the criteria of Feinberg and Floyd [271] with the exception that, for the last sleep cycle, no minimum REM-sleep duration was required. Thereafter, each sleep cycle was divided into 10 equal time intervals during NREM

sleep and 4 equal time intervals during REM sleep. To investigate the decay of SWA (0.75-4.5 Hz) during NREM sleep, an established electrophysiological correlate of the decrease of homeostatic sleep pressure, across the baseline and the recovery nights in both protocols the following exponential decay function was fitted to the individual data during the NREM episodes of each group: $SWA_t = SWA_\infty + SWA_0 * e(-rt)$; with SWA_t = averaged SWA per sleep cycle, SWA_0 = intercept on the y axis, SWA_∞= horizontal asymptote for time t = ∞, r = slope of the decay, t = average timing of the NREM cycle midpoint.

The 10 nap episodes were categorised either as a biological day or biological night nap in order to compare diurnal and nocturnal EEG sleep spectra during stage 2 sleep. A nap was considered as day nap if the start time of the nap was between the melatonin downward and upward crossing time (see methods section below). Subsequently, a nap qualified for the biological night occured during the main melatonin secretion episode between the upward and downward mean crossing time. The average number of biological day and night naps per group was 7.2 ± 0.67 and 2.8 ± 0.67 for young depressed, 7.6 ± 0.52 and 2.4 ± 0.52 for healthy young, and 7.8 ± 0.46 and 2.1 ± 0.64 for healthy older women. These numbers did not significantly differ between groups (p at least > 0.05). Stage 2 sleep duration was not significantly different between groups and biological day and night according to the interaction effect of the factors group and biological timing (day vs night naps) of a 2-way rANOVA ($F_{2,22} = 0.22$; $p > 0.8$). Moreover, 1-way rAnova performed for each group seperately showed no significant difference of stage 2 sleep duration between the biological day and night (p at least > 0.4).

4.2.4 Salivary melatonin sampling and subjective sleepiness ratings

Saliva collections were taken every 30 minutes during scheduled wakefulness during the entire 40-h protocol. A direct double-antibody radioimmunoassay (RIA) was used for the melatonin assay which was validated by gas chromatography–mass spectroscopy with an analytical least detectable dose of 0.65 pm/ml (Bühlmann Laboratories, Schönenbuch, Switzerland) [272]. All melatonin values were collapsed into 1.25-h bins per subject before averaging across groups whereby missing values were linearly interpolated. Melatonin, as one of the most reliable endogenous circadian phase measures [298, 299], was used to estimate circadian timing and its phase relationship with sleep phase preference. Thereby, upward and downward mean crossing times of the 24 hour mean, the average level of melatonin and the respective secretion duration in between these mean crossing times were calculated for each subject before averaging across groups according to Knoblauch et al. [300] and Münch et al. [278](see Tab. 4.4).

Study participants rated their sleepiness and mood every 30 min during scheduled wakefulness across the 40-h study protocol by means of questionnaires. Sleepiness was assessed by the Karolinska Sleepiness Scale (KSS) with a rating range from 1 (very alert) to 9 (very

sleepy) [274]. KSS values were collapsed into 1.25-h bins per subject before averaging across groups.

4.2.5 Statistical analysis

The statistical packages SAS® (SAS Institute, Inc.; Version 9.1.3) and Statistica® (StatSoft Inc., STATISTICA for windows, Version 8.0) were used. Sleep stages were analysed with repeated-measures ANOVA (PROC GLM) and p values were based on Huynh-Feldt corrected degrees of freedom. Post-hoc comparisons on sleep stages were based on Duncan's multiple range test and the levels of significance of these post-hoc comparisons were adjusted according to the false discovery rate procedure [275]. Comparisons of EEG power density, melatonin and subjective sleepiness were made with mixed-model rANOVA (PROC MIXED) and p values were based on Kenward-Roger's corrected degrees of freedom [276]. Contrasts were assessed with the LSMEANS statement and the respective level of significance was adjusted according to the Tukey-Kramer method [277]. EEG power density was averaged during NREM sleep per group for the baseline and the recovery nights and expressed as percentage of the respective values of the healthy young group (100%). 1-, 2-, 3- and 4-way mixed-model rANOVAs were used with the categorical factor group (young depressed vs healthy young vs healthy older women) and the repetitive factors derivation, night, and time interval (e.g. nap, sleep cycle etc.).

4.3 Results

4.3.1 Sleep stages during baseline and recovery nights

Sleep parameters of the baseline and recovery nights for young volunteers with major depressive disorder (MDD), healthy young (HY) and healthy older (HO) women are summarized in Table 4.1. A two-way rAnova disclosed significant differences between groups for total sleep time (TST), sleep efficiency (SE), wake after lights off (WALO), wake after sleep onset (WASO), stage 1, stage 4, and slow-wave sleep (SWS). Post-hoc comparisons for the significant group effects showed significant less TST, lower SE, higher WALO and WASO values, and shorter stage 4 duration for the older volunteers compared to both, the young healthy and young depressed women (p at least < 0.05). SWS in MDD but not in HY was significantly higher compared to HO ($p < 0.01$). Furthermore, MDD had significantly less stage 1 sleep than HO and HY ($p < 0.01$).

The nap protocol resulted in significant differences between the baseline and the recovery nights for the factors TST, SE, NREM sleep ($p < 0.01$), WALO, stage 1 and stage 2 sleep latency ($p < 0.001$), movement time, stage 1 ($p < 0.01$), and WASO ($p<0.05$). While TST, SE, and NREM sleep proportion decreased in the recovery night compared to the baseline night, the wake period after lights off and the latency to sleep onset increased.

Table 4.1: Sleep parameters of the baseline and recovery nights based on visual scoring for all groups

Parameter	young depressed women		healthy young women		healthy older women		2-way rAnova					
	baseline	recovery	baseline	recovery	baseline	recovery	group		night		group x night	
							p	$F_{2,22}$	p	$F_{1,22}$	p	$F_{2,22}$
TST, min	449.9 ± 21.0	413.2 ± 26.2	449.5 ± 17.1	412.2 ± 53.9	403.2 ± 47.9	361.6 ± 54.5	**	6.05	***	19.35	-	0.03
SE, %	94.8 ± 1.5	86.8 ± 4.8	93.6 ± 3.5	85.9 ± 11.3	84 ± 10	75.4 ± 11.4	**	6.81	***	23.14	-	0.02
WALO, %	3.3 ± 1.2	10.7 ± 5.2	3.7 ± 2.1	10.4 ± 9.3	14.4 ± 9.7	21.1 ± 10.4	**	8.93	***	18.05	-	0.02
WASO, %	2.0 ± 1.8	5.5 ± 6.7	2.0 ± 2.1	8.0 ± 12.4	16.1 ± 12.3	19.7 ± 12.8	**	8.57	*	4.82	-	0.17
SL1, min	6.4 ± 5.2	25.9 ± 15.8	8.9 ± 4.0	19.8 ± 11.8	8.3 ± 3.9	27.8 ± 17.5	-	0.31	***	44.04	-	1.28
SL2, min	9.3 ± 6.1	31.6 ± 20.2	15.7 ± 8.1	30.5 ± 15.9	12.2 ± 5.6	43.1 ± 30.7	-	0.63	***	29.1	-	1.18
RL, min	85.4 ± 36.6	85.0 ± 23.3	71.9 ± 10.8	90.9 ± 45.4	104 ± 37.5	101.4 ± 36.9	-	1.57	-	0.38	-	0.62
MT, %	0.7 ± 0.2	0.9 ± 0.4	0.9 ± 0.6	1.4 ± 1.2	0.6 ± 0.3	1.3 ± 1.1	-	1.13	**	12.04	-	1.17
St1, %	6.9 ± 2.7	8.5 ± 3.2	11.1 ± 4.1	14.5 ± 4.8	11.9 ± 3.4	14.1 ± 5.3	**	6.13	**	10.15	-	0.53
St2, %	51.1 ± 3.7	51.4 ± 6.2	49.5 ± 4.7	49.7 ± 5.5	60.8 ± 11.4	55.0 ± 12.3	-	2.98	-	1.9	-	2.32
St3, %	11.1 ± 3.6	9.9 ± 3.8	11.2 ± 5.1	10.0 ± 3.1	9.2 ± 5.0	8.2 ± 4.9	-	0.56	-	3	-	0.01
St4,%	10.9 ± 7.3	11.0 ± 5.8	8.6 ± 4.5	6.7 ± 4.3	2.3 ± 2.5	2.5 ± 3.1	**	7.86	-	0.37	-	0.65
SWS, %	21.9 ± 8.1	21.0 ± 6.7	19.8 ± 7.3	16.7 ± 6.7	11.4 ± 7.1	10.7 ± 7.6	*	5.09	-	2.27	-	0.49
NREM, %	73.1 ± 5.3	72.4 ± 4.4	69.3 ± 5.1	66.3 ± 4.0	72.2 ± 8.0	65.8 ± 8.1	-	2	**	9.04	-	2.22
REM, %	20.0 ± 3.3	19.1 ± 2.5	19.7 ± 3.6	19.2 ± 3.4	15.9 ± 5.7	20.2 ± 5.4	-	0.52	-	0.97	-	2.79

*Sleep parameters are averaged separately across the baseline night and the recovery night (mean ± SD) for young women with major depression (MDD; n=9), young healthy women (HY; n=8), and older healthy women (HO; n=8). TIB = total time in bed (from lights off to lights on); TST = total sleep time; SE = sleep efficiency (TST / TIB * 100); WALO = wake after lights off (in % of TIB); WASO = wake after sleep onset (in % of TST); SL1 = sleep latency to stage 1; SL2 = sleep latency to stage 2; RL = REM latency; MT = movement time after sleep onset (in % of TST); St1-St4 = sleep stages 1-4 (in % of TST); SWS = slow-wave sleep (sum of stages 3 and 4 in % of TST); NREM = non-REM sleep (sum of sleep stages 2-4 in % of TST); REM = REM sleep in % of TST; *p < 0.05; **p < 0.01; ***p < 0.001.*

4.3.1.1 Nap sleep episodes with reference to the baseline night

Sleep parameters for the nap sleep episodes are shown in Table 4.2 (summarized values accross the 10 nap episodes expressed as percentage of the TST during the baseline night). Compared with the baseline night the summarized TST during the nap episodes distributed over the 40-h protocol accounted approximately for 1 night of sleep during baseline night. One-way Anova with the main factor group disclosed no significant differences for TST, SE, stage 1, NREM, and REM sleep durations between the three groups. In contrast, stage 2 sleep duration differed significantly between the groups ($p < 0.01$) in such a way that older women showed significantly more stage 2 sleep compared to both young groups ($p < 0.01$). Moreover, healthy young women exhibited significant more stage 3 sleep compared to healthy older women ($p < 0.05$, Duncan's multiple range test corrected for multiple comparisons). Young depressed women showed significantly more stage 4 sleep compared to HO ($p < 0.001$) and HY ($p < 0.05$). HO showed significantly less SWS compared to HY and MDD ($p < 0.01$). Total sleep duration across the 10 naps and the baseline night did

not differ significantly within the groups ($p > 0.05$, t-test for dependent samples). Sleep efficiency during the naps was significantly lower for all groups when compared to baseline sleep values (p at least < 0.001; t-test for dependent samples).

Table 4.2: Sleep parameters based on visual scoring during 10 naps expressed as a percentage of the baseline night

Parameter	MDD	HY	HO	1-way Anova		post-hoc analysis		
				group		MDD vs HY	MDD vs HO	HY vs HO
				p	$F_{2,22}$			
TST, %	95.8 ± 21.7	104.4 ± 23.2	105.1 ± 16	-	0.543	-	-	-
SE, %	66.5 ± 10.3	72.3 ± 8.6	58.7 ± 15	-	2.77	-	-	-
St1	13.1 ± 5.8	19.5 ± 10.2	19.8 ± 6.5	-	2.09	-	-	-
St2	43.6 ± 12.3	47.1 ± 12.7	67.4 ± 16.1	**	7.21	-	**	**
St3	9.8 ± 4.1	14.0 ± 4.3	8.0 ± 5	*	3.95	-	-	*
St4	16.2 ± 9.6	8.7 ± 5.9	2.0 ± 3.2	**	8.95	*	***	-
SWS	26.0 ± 10.1	22.7 ± 6.9	10.0 ± 7.7	**	8.25	-	**	**
NREM	69.6 ± 19.3	69.8 ± 15	77.4 ± 12	-	0.64	-	-	-
REM	13.1 ± 4.4	15.1 ± 7.4	8.0 ± 6.8	-	2.75	-	-	-

*All sleep parameters except SE are expressed as a percentage of the TST during the baseline night (mean ± SD) for young depressed (MDD; n=9), young healthy (HY; n=8), and older healthy women (HO; n=8). SE = TST 10 naps/total time in bed during 10 naps*100. P values of a 1-way Anova and of the post-hoc comparisons based on the Duncan's multiple range test are indicated (*p < 0.05; **p < 0.01; ***p < 0.001).*

In order to inspect the time course of sleep stages 1-4, REM, and wakefulness across the 10 nap episodes an area diagram was calculated which displays for each nap and group the averaged relative time proportion of selected sleep stages and wakefulness as a percentage of the total nap duration (Figure 4.3.1). A 2-way rAnova on relative sleep stage values disclosed significant differences between groups with a higher sleep stage 4 proportion in young depressed compared to healthy young and healthy older women ($p < 0.05$ and $p < 0.001$ respectively; Curran-Everett adjusted p values). Furthermore, healthy older women showed significantly more stage 2 sleep than the other two groups across the 40-h nap protocol ($p < 0.01$). The time course of all selected sleep stages, wakefulness, and of the sleep efficiency (SE) varyied significantly ($p < 0.001$). No group differences for the relative time proportions of sleep stages 1, 3, REM, wakefulness, and SE nor any significant interaction of the factors group*nap time were observed.

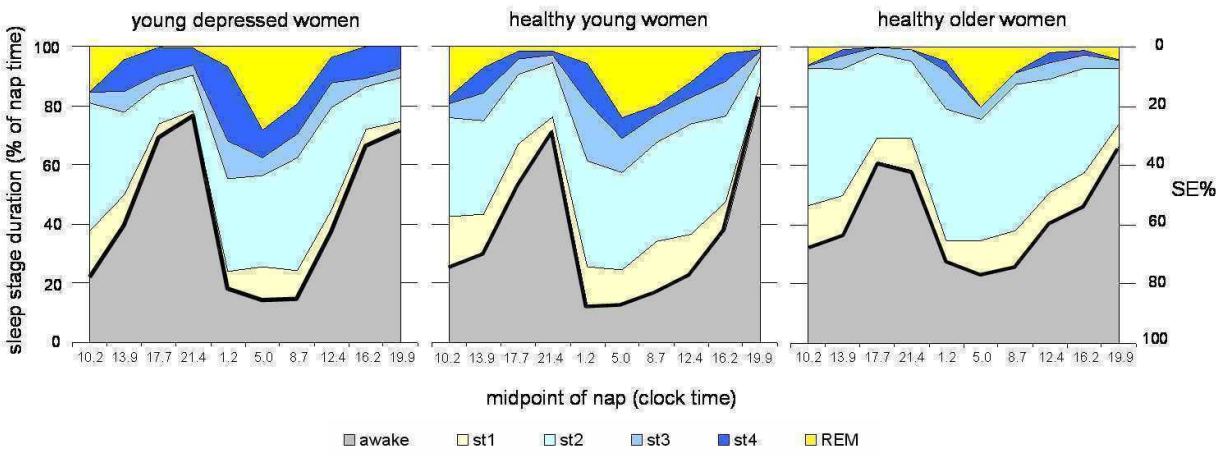

Figure 4.3.1: Time course of relative sleep stage proportions per nap across the 40-h protocol

The *figure displays the mean duration of different sleep stages and wakefulness expressed as % of total nap duration (75 minutes) per nap and group. Stage durations are derived from visually scored sleep stages. St1-st4 = sleep stages 1-4. Black bold line indicates the time course of sleep efficiency (SE) per nap (total sleep time expressed as % of the total nap time) on an inverted scale as indicated by the right ordinate.*

Furthermore, a 2-way rAnova further disclosed a significant effect of the factor time of day for total sleep time (TST) as well as for REM, stage 1, and stage 2 sleep latencies ($p < 0.001$). Group differences were only significant for the REM sleep latency with longer values in healthy older women compared to the two young groups (p at least < 0.05). No significant interactions of the factors group*time of day were observed ($p > 0.1$).

4.3.2 NREM EEG power activity during baseline and recovery nights

Figure 4.3.2 illustrates the relative EEG spectra during baseline and recovery nights for young depressed (MDD) and healthy older (HO) women expressed as percentage of the healthy young (HY) women (100%) during NREM sleep in the frequency range of 0.75 and 25 Hz for F, C, P, and O derivations. Mixed-model 3-way rANOVA (on absolute EEG spectral values) disclosed a significant difference for the main factor group ($p < 0.05$) in the delta and for one frequency bin in the theta range (0.75-4.5 Hz and 4.75 Hz) as well as in the sigma range (12.5-14 Hz). Post-hoc inspection of the data showed significant higher EEG power of MDD than HY for some of the frequency bins in the delta range (1.5-2.5 Hz) and than HO in the delta range and in some of the frequency bins of the theta and sigma range (0.75-4.5 Hz, 4.75 Hz, and 12.75-13.5 Hz) ($p < 0.05$; LSMEANS procedure, Tukey-Kramer adjusted). Furthermore, HY showed higher EEG power than HO for one of the frequency bins in the theta range and in the sigma range (0.75 Hz and 12.75-14 Hz) ($p < 0.05$). The significant range overlap of young depressed women with the other two groups in the delta frequencies 1.5-2.5 Hz was chosen as the range for subsequent analysis (e.g. EEG spectra analysis and delta activity analysis per sleep cycle).

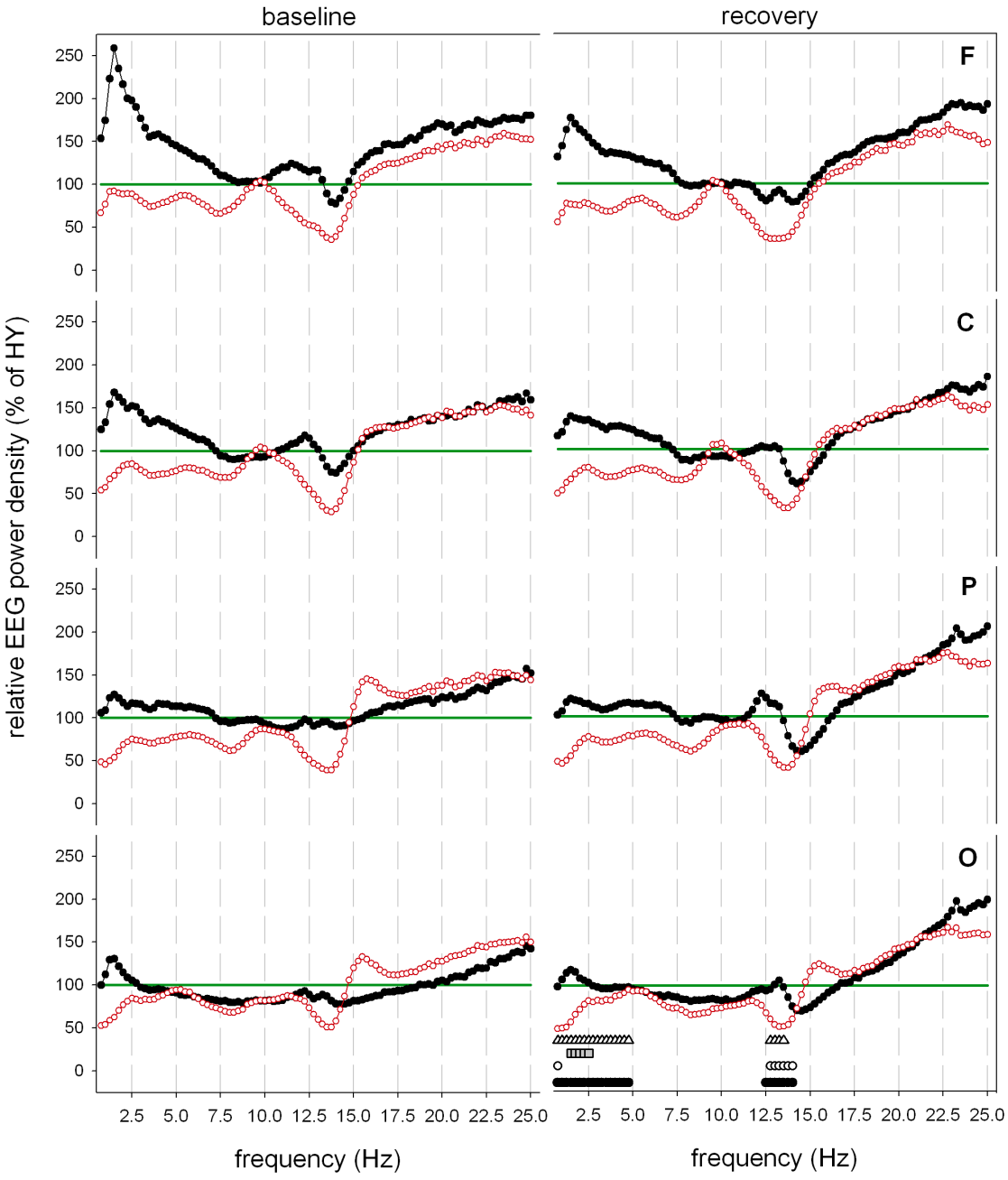

Figure 4.3.2: Relative NREM EEG spectra during baseline and recovery nights

Relative EEG spectra values are shown for 0.75-25 Hz for F, C, P, and O derivations (collapsed left, central, and right values for Fz, F3, F4, Cz, C3, C4, Pz, P3, P4, and Oz, 01, O2 respectively) for healthy young (HY; green reference line at 100%; n=8), healhy older (HO; in % of HY; red open circles; n=8), and major depressed (MDD; in % of HY; black filled circles; n=8) women. Significant differences for the factor group (filled black circles) are indicated as well as respective post-hoc comparisons: open circles = HY > HO; shaded square = MDD > HY; open triangle up = MDD > HO (statistics are based on the absolute EEG spectra values, mixed-model 3-way rAnova, post-hoc analysis with LSMEANS procedure, Tukey-Kramer adjusted p values).

Mixed-model 3-way rAnova showed significant differences for the main factor derivation for all frequency bins except for the theta frequencies 6.25 and 6.5 Hz. A post-hoc anal-

ysis yielded a significant frontal predominance compared to central, parietal and occipital derivations in the delta frequency range of 0.75-2.25 Hz. In higher frequencies no significant difference between frontal and central derivations but between parietal and occipital derivations occured. The interaction of the main factors group*derivation yielded significance for the frequency bins in the delta range (0.75-4.5 Hz), some of the frequency bins in the theta range (4.75-7 Hz), in the alpha and sigma range (11.5-12.25 Hz, 13.25-14.25 Hz, 1525-16 Hz), and in the beta range (16.25-21.5 Hz, 22-22.5 Hz) ($p < 0.05$). Post-hoc analysis yielded higher frontal values in MDD than in HY and HO in frequency bins of the delta range (1-3 Hz) as well as higher values in MDD than in HY in the beta frequency bins (18.25-20 Hz) ($p < 0.05$, LSMEANS procedure with Tukey-Kramer adjusted p values).

There was no significant interaction of the factors group*night but the interaction der*night yielded significance in the theta frequency bin 5 Hz, for frequency bins in the alpha range (11.25-12 Hz), for frequency bins in the sigma spindle range (13.5-15.5 Hz) and the beta frequency range (22.75-23 Hz, 23.75 Hz, and 24.75 Hz). Thereby, significant higher values in fronto-central derivations were found in the theta 5 Hz bin during the recovery night compared to the baseline night, higher frontal values during the recovery compared to the baseline night for the alpha frequencies 11.25-12 Hz, higher central and parietal values for the sigma spindle range (13.5-15.5 Hz), and higher values in all derivations during the recovery night compared to the baseline night for the beta frequency bins 22.75-23 Hz, 23.75 Hz, and 24.75 Hz ($p < 0.05$). Finally, 3-way mixed model rAnova yielded significance for the interaction group*night*derivation in the sigma spindle range (14.75-15.75 Hz; $p<0.05$) with no significant differences between groups per night and derivation after Tukey-Kramer adjustment of the p values of the post-hoc analysis.

Subsequently, relative NREM spectra per sleep cycle (recovery night EEG spectra per cycle as a percentage of respective baseline values per subject averaged across groups) and group were calculated (see Figure 4.3.3). Mixed-model 3-way rAnova with the factors group, cycle, and derivation showed significant interaction of the factors group*cycle with higher values of HY than MDD in NREM sleep cycle 3 in some of the delta and theta frequency bins (1-2 Hz, 2.75 Hz, 3.25 Hz, 4 Hz, and 5-5.25 Hz; $p < 0.05$). Furthermore, relative EEG power density during NREM sleep cycles 1 and 2 were significantly lower than during sleep cycle 3 in HY in the delta and theta frequency bins 1-5.75 Hz ($p < 0.01$).

There were no significant differences of EEG power density between NREM sleep cycles 1-3 in MDD and HO (post-hoc comparisons within each group, $p > 0.05$) as well as between these two groups per cycle (post-hoc comparison between groups, $p > 0.05$). Furthermore, mixed-model 3-way rAnova showed significant interaction of the factors group*cycle*derivation for some frequency bins of the delta, theta, alpha, sigma, and beta range (4.25-5 Hz, 5.5-7 Hz, 10-10.25 Hz, and 15.25-16.25 Hz; $p < 0.05$).

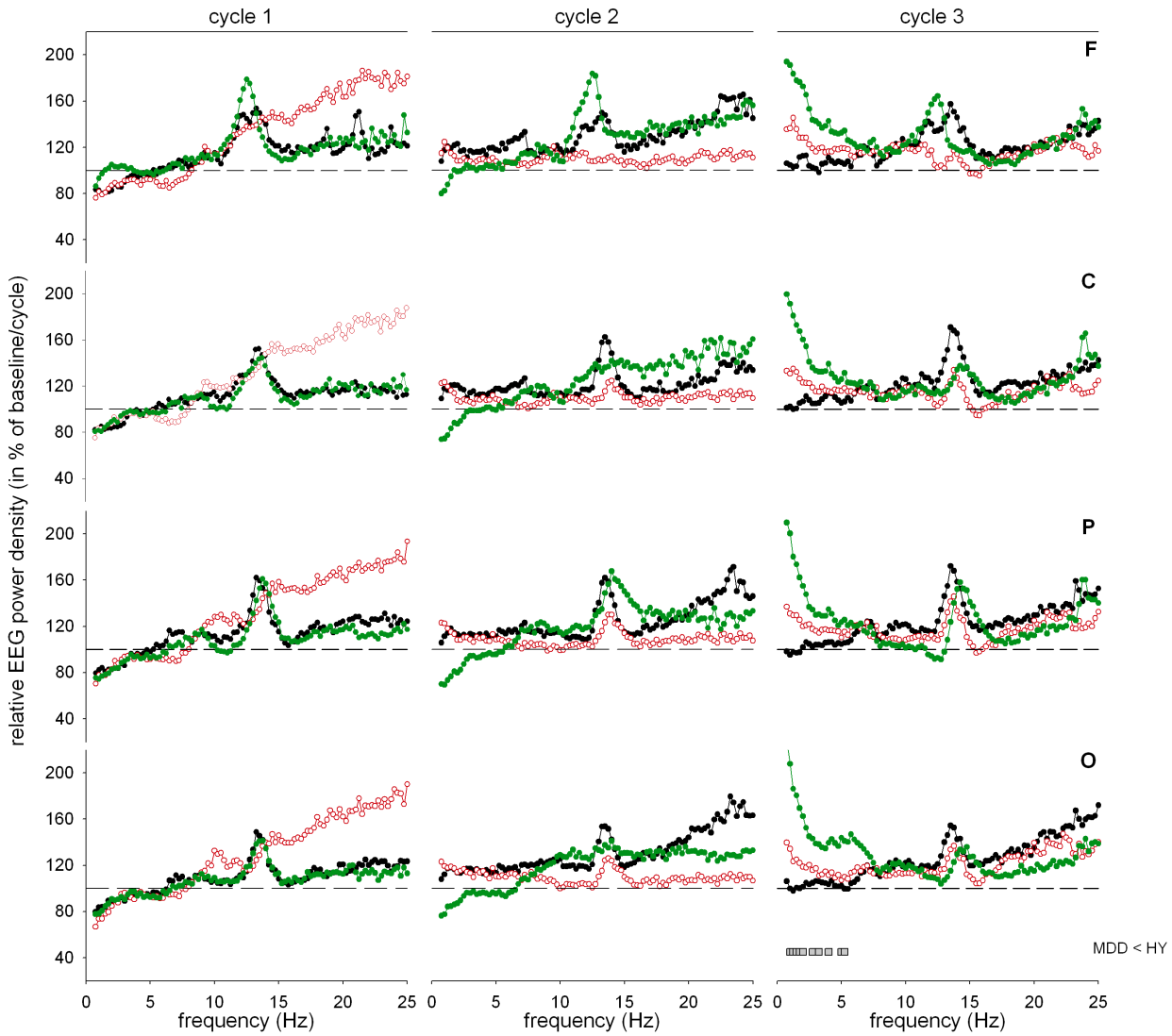

Figure 4.3.3: Relative EEG power density during the recovery night for NREM sleep cycles 1-3 between 0.75-25 Hz

*The figure illustrates relative EEG spectra values (recovery night in % of baseline night per cycle) for NREM cycles 1-3 for F, C, P, and O derivations between 0.75 and 25 Hz for healthy young (HY; green filled circles; n=8), healthy older (HO; red open circles; n=7), and young depressed (MDD; black filled circles; n=7) women. Significant post-hoc comparisons for the interaction of the factors group*cycle are indicated near the abscissae (gray shaded squares = MDD < HY; $p < 0.05$).*

In order to analyse the time course of the EEG delta power in detail, percentiles (see methods section) in the frequency range between 1.5 and 2.5 Hz (= significant EEG band between MDD and the other two groups accross baseline and recovery nights as illustrated in Fig. 4.3.2) were calculated as a percentage of the mean delta activity during baseline night for NREM sleep cycles 1-3 for F, C, P, and O derivations (see Fig. 4.3.4).

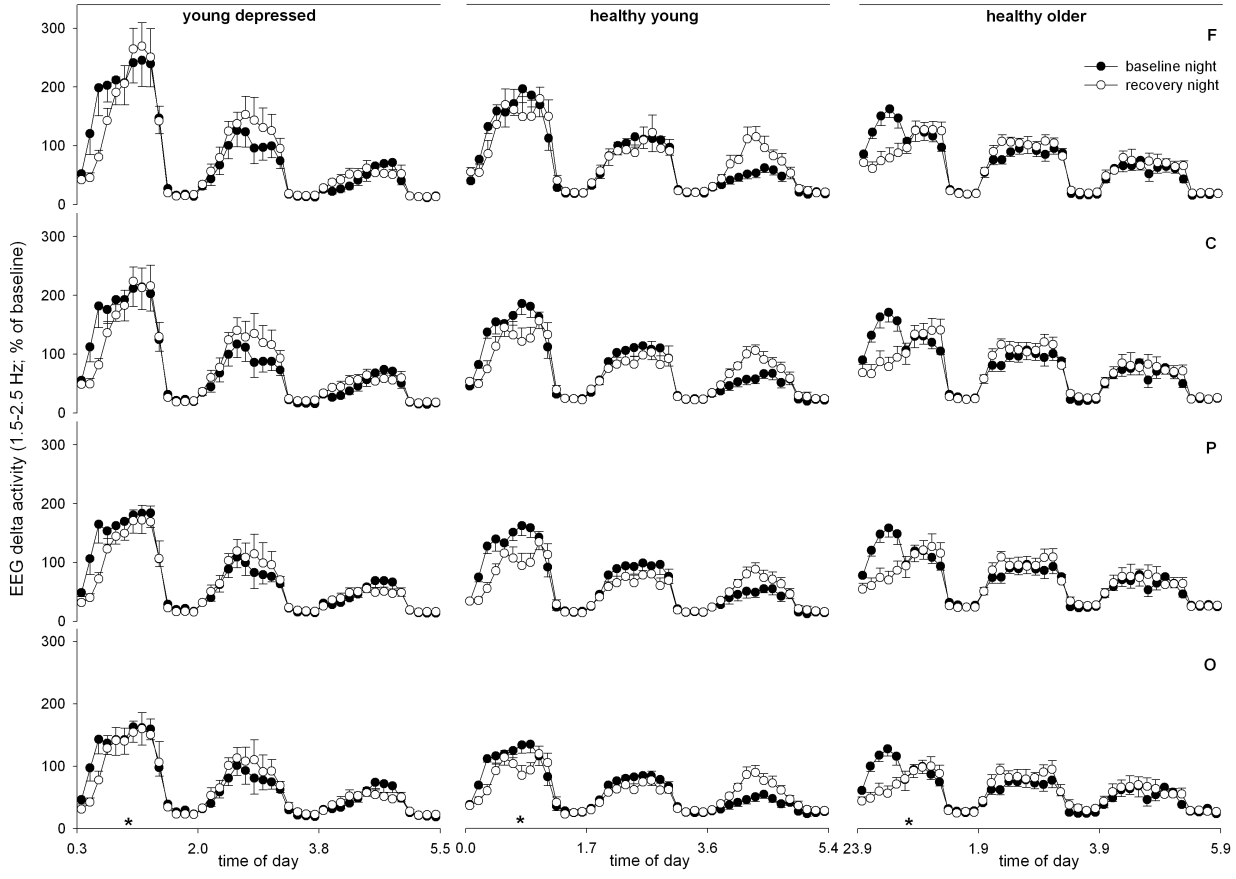

Figure 4.3.4: EEG delta power activity for NREM-REM sleep cycles 1-3

EEG delta activity (1.5-2.5 Hz) per sleep cycle 1-3 for collapsed derivations (F, C, P, O) is expressed for each group separately as a percentage of the respective baseline value (young depressed, n = 7; healthy elderly, n = 7; healthy young, n=8; mean ± SEM). Black filled circles = baseline night, black open circles = recovery night. Asterisks indicate significant post-hoc comparisons of mean NREM EEG values per cycle between baseline and recovery night within each group (p<0.05; LSMEANS procedure, Tukey-Kramer adjusted).

A mixed-model 4-way rAnova disclosed no significant effect of the main factors group and night but of the factors derivation and cycle ($p < 0.001$). Furthermore, significant interactions of the factors group*night*cycle ($p < 0.001$) and group*night*cycle*derivation ($p < 0.001$) were observed. Significant post-hoc comparisons for the 3-way interaction are displayed in Figure 4.3.4 and show significant lower EEG delta power during the first NREM-REM sleep cycle in the recovery night compared to the baseline night within each of the three groups ($p < 0.05$; LSMEANS procedure). There was no difference of the duration of the NREM-REM cycles 1-3 between the three groups during the baseline or recovery night

(p>0.6, mixed-model 2-way rANOVA; data not shown).

4.3.3 Decay of EEG slow-wave activity during baseline and recovery nights

The decay of EEG delta power for each group during baseline and recovery nights was analysed by fitting a nonlinear regression function (see methods section) to the mean SWA values centred at the middle of each sleep cycle of each subject per group in the relative EEG delta range (1.5-2.5 Hz; percentage of baseline night). The fitted exponential decay function for all three groups during the baseline and recovery nights for the collapsed frontal derivations is illustrated in supplemental figure S1. The respective estimated parameters of the decay function for the frontal derivations are displayed in Table 4.3.

Although the decay of slow-wave activity of young depressed women was higher during recovery night compared to the respective slopes of healthy young and healthy older women the difference was not significant since the mean decay rates of each group reached the 95% confidence interval of the other two groups during baseline night and during recovery night. Furthermore, mean values of the baseline slopes overlapped with the 95% confidence interval of recovery nights within the groups.

Table 4.3: Estimated nonlinear regression parameters for the decay of relative EEG delta power activity during baseline and recovery nights

parameter	young depressed women		healthy young women		healthy older women	
	baseline night	recovery night	baseline night	recovery night	baseline night	recovery night
Decay rate /min	0.006 ±0.0029	0.0054 ±0.0029	0.0069 ±0.0025	0.0036 ±0.0059	0.005 ±0.0028	0.0028 ±0.0068
95% CI	$4.8*10^{-5}$-0.012	-0.00065-0.0114	0.00175-0.0119	-0.00785-0.0151	-0.0007-0.0108	-0.0112-0.0167
R	0.85	0.86	0.89	0.61	0.84	0.56

The estimated paramters of the nonlinear regression analysis for the decay of EEG delta power during baseline and recovery nights for all groups are indicated for the collapsed frontal derivations during all NREM sleep episodes (mean of all cycles ± SEM). 95% CI = 95% confidence interval; R = index for goodness of fit.

4.3.4 EEG spectra during nap episodes

Nap sleep episodes were grouped according to their occurence either during the biological day or biological night (see methods section). The respective mean EEG spectra during stage 2 sleep in the frequency range 0.75-25 Hz for F, C, P, and O derivations for all three groups are displayed in supplemental figure S2. Mixed-model 3-way rAnova disclosed significant effects of the factor group with higher values for major depressed women (MDD) compared to healthy young women (HY) in the beta range (17.75-18 Hz, 18.5-18.75 Hz,

19.25-20 Hz, 20.5-22 Hz, and 22.5-23 Hz; $p < 0.05$) and compared to healthy older women (HO) in the delta, theta, and in the sigma range (1.5-5.5 Hz and 13-14.25 Hz; $p < 0.05$). Furthermore, higher spectra values of HY compared to HO occured in the theta and sigma range (6.75-7.75 Hz and 12.5-14.5 Hz; $p < 0.05$). A significant interaction between the factors group*condition*derivation occured in some of the frequency bins of the delta, theta, alpha, sigma, and beta ranges (0.75-1 Hz, 1.75 Hz, 6-6.5 Hz, 7.25-9 Hz, 9.5-12.25 Hz, 13.25-14 Hz, 14.75-18 Hz; $p<0.05$) whereby for the delta bin 1.75 Hz significant higher diurnal spectra values occured in MDD than in HY for the frontal derivations ($p<0.01$). The interaction group*derivation was significant for the frontal derivations with higher spectra values for MDD than HY (delta frequency bins 1.75-2.25 Hz, $p < 0.01$; beta range 17.5-20 Hz and 20.5-20.75 Hz; $p<0.05$) and than HO (delta range 2-4.25 Hz and sigma range 11.75-1325 Hz; $p < 0.05$). Moreover, in central derivations higher values of MDD compared to HO occured in some frequency bins of the delta and theta range (2.5-5.5 Hz) and the sigma range 13.5-14 Hz ($p<0.05$) as well as higher values of HY than HO in the sigma range (13.5-14.5 Hz). Additionally, post-hoc analysis on the interaction group*derivation showed higher spectra value of healthy young compared to healthy older women for the parietal derivations in the sigma frequency range 13.75-14.25 Hz ($p < 0.05$; LSMEANS statement, Tukey-Kramer adjusted).

The differences between biological day and night spectra between the three groups were significant in the frequencies 7.75 Hz, 8.25 Hz, 10.5-12.5 Hz, 13.25-13.75 Hz, 14.75-15.25 Hz (significant interaction of the factors group*condition; $p<0.05$). Post-hoc analysis thereby showed significant lower nocturnal values of healthy older than healthy young women in the alpha frequency bin 8.25 Hz and the sigma frequency bins 12.5 Hz and 13.25-13.75 Hz as well as compared to young depressed women in the sigma frequency bin 13.25 Hz ($p<0.05$; LSMEANS statement). Healthy young women showed higher values during the biological night compared to day values in the frequency bins 12.25-12.5 Hz and 13.25-13.75 Hz. Furthermore, HY as well as MDD showed higher EEG spectra values during the biological day compared to the biological night in the frequency bins 14.75-15.25 Hz ($p < 0.05$).

Relative EEG values during the biological night stage 2 sleep as a percentage of respective values during the biological day are presented in Figure 4.3.5. Young depressed women had lower relative EEG values compared to healthy young women in some of the frequency bins of the alpha and sigma range (10.5-11.5 Hz and 13.5-13.75 Hz)($p < 0.05$). Furthermore, healthy older women exhibited significant lower relative EEG values during the biological night in some frequency bins of the theta, alpha, and sigma range compared to healthy young (7.75-8.75 Hz and 10.5-13.75 Hz) and young depressed women (7.75-8.5 Hz and 11.75-13.5 Hz)($p<0.05$) whereas in the 15 Hz frequency bin HO showed higher EEG values than the two young groups. Importantly, at 13.5 Hz there was a significant difference between all three groups ($p < 0.05$). Furthermore, mixed-model 2-way rAnova disclosed significant interaction of the factors group*derivation in the frequency bins 1-3 Hz, 6-6.25 Hz, 8-9 Hz, 9.5 Hz, 10 Hz, 10.5-12.25 Hz, 13.5-13.75 Hz, and 15.25 Hz ($p < 0.05$).

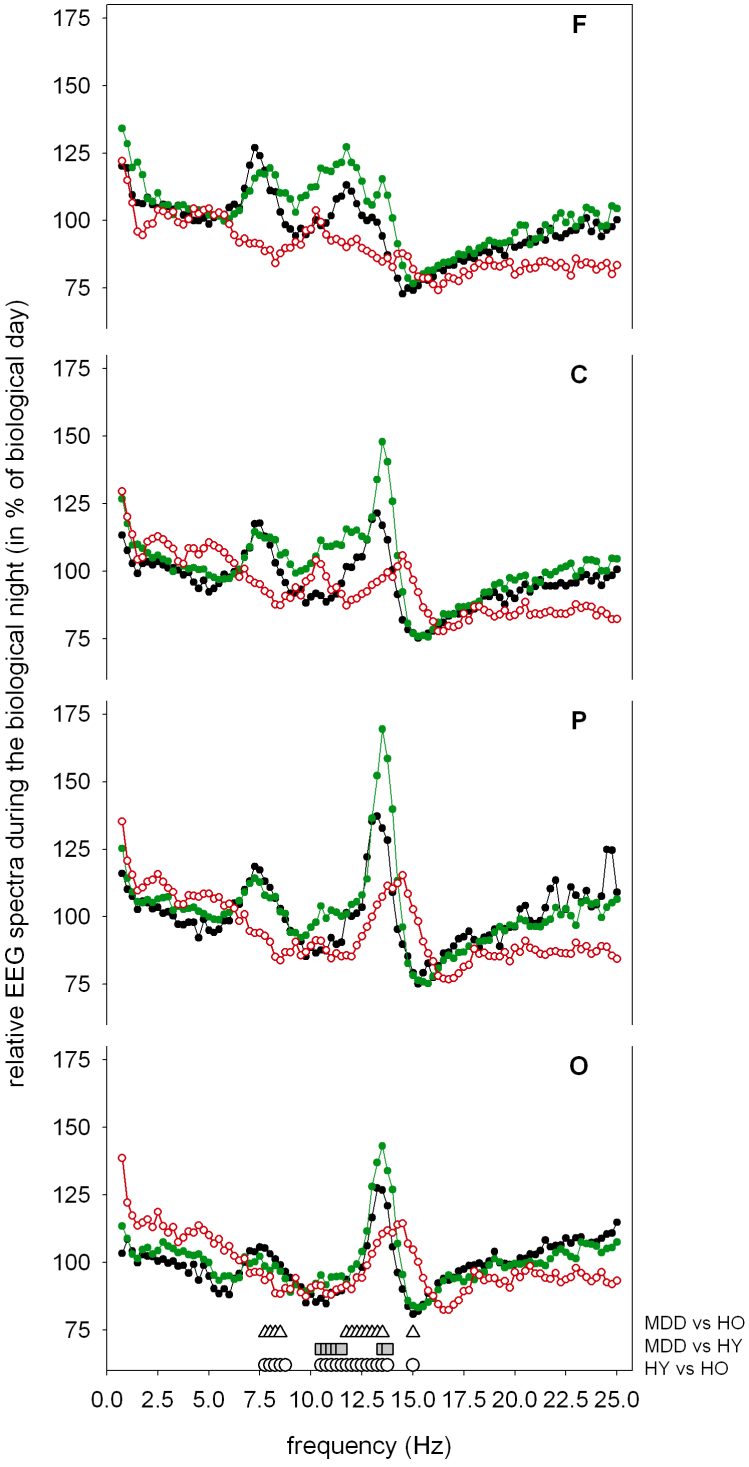

Figure 4.3.5: Relative EEG spectra during the biological night

The figure illustrates relative EEG spectra during the biological night as a percentage of the respective biological day values (all EEG spectra were analyzed during stage 2 sleep). Post-hoc analysis on significant differences between the three groups are indicated near the abscissa (p at least < 0.05; LSMEANS statement, Tukey-Kramer adjusted). Young depressed women (MDD, n=9) = black filled circles; healthy young women (HY, n=8) = green filled circles; healthy older women (HO, n=8) = red open circles.

Post-hoc analysis on this interaction based on LSMEANS statement showed no significance,

hence group differences were not dependent on derivation although they seem to be most pronounced in parietal derivations according to visual inspection of figure 4.3.5.

4.3.5 Circadian variables

Salivary melatonin, as a robust circadian rhythm marker, sampled every 30 minutes over the entire 40 hours protocol was collapsed into 1.25 hour bins (Figure 4.3.6, top panel). Mixed-model 2-way rAnova disclosed significant effects of the main factors group ($p < 0.05$) and session ($p < 0.001$) as well as of their interaction ($p < 0.001$). Post-hoc analysis showed signficant higher melatonin values of healthy young (HY) compared to healthy older (HO) women during two night sessions ($p < 0.05$). No significant differences of young depressed (MDD) women compared to the other two groups were observed.

The time course of mean subjective sleepiness per group is illustrated in Figure 4.3.6 (lower panel). The ratings were derived from the Karolinska Sleepiness Scale (KSS) and as with melatonin values collapsed into 1.25 hour bins. MDD exhibited significant higher subjective sleepiness than the other two groups ($p < 0.01$). Furthermore, the analysis showed significance for the factor session ($p < 0.001$) and a tendency to significance for the interaction group*session ($p = 0.054$).

Different phase positions and the timing of the melatonin secretion over the 40-h protocol were calculated for each subject and subsequently averaged across groups (Table 4.4). The three groups neither differed significantly in the upward nor the downward mean crossing time of melatonin ($p > 0.1$, t-test for independent samples). Similarly, no significant group differences occured with reference to the temporal midpoint of the melatonin peak, mean bedtime clock hours, and the circadian phase angles (expressed as differences between habitual bedtime and the upward or downward mean crossing time, respectively) ($p > 0.1$). Healthy young participants exhibited a higher mean amount of melatonin secreted between the upward and downward mean crossing time compared to healthy older ($p < 0.05$), and young depressed women had a significantly longer duration of melatonin secretion between the upward and downward crossing time than healthy older women ($p < 0.05$).

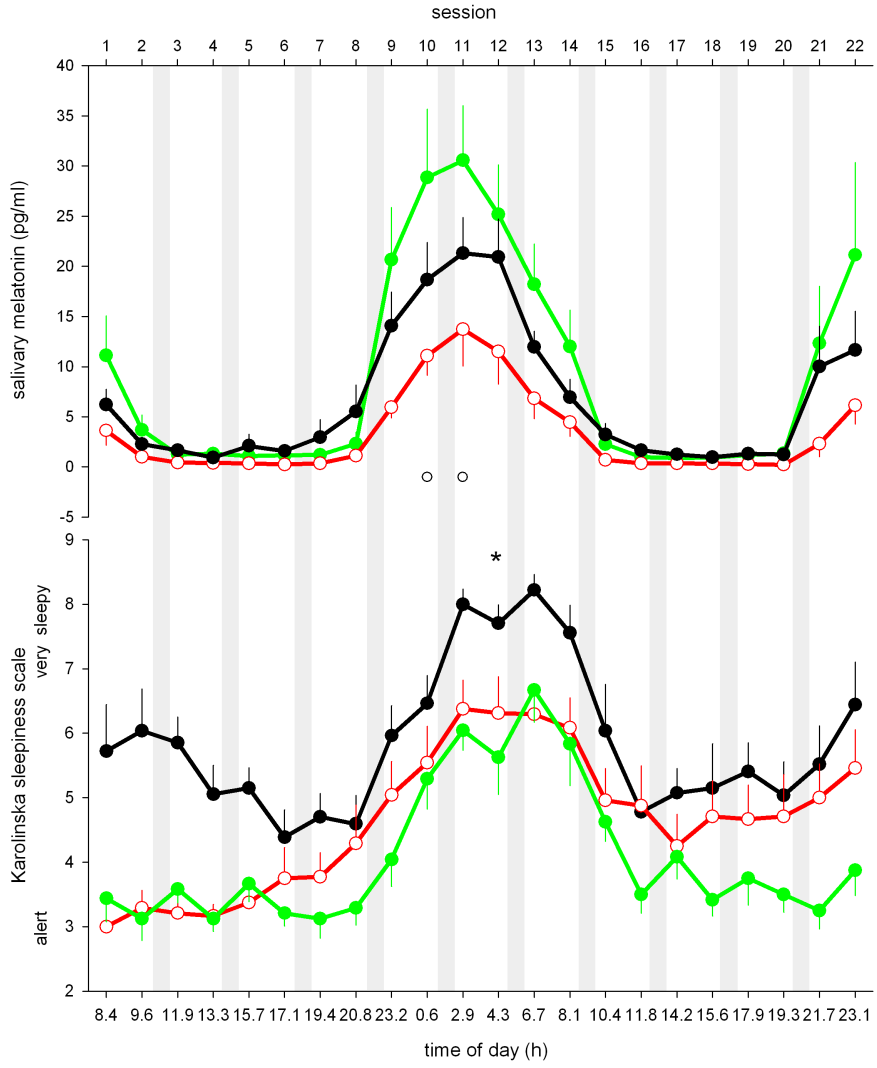

Figure 4.3.6: Mean salivary melatonin levels and subjective sleepiness ratings during the 40-h nap protocol

*The top panel displays mean salivary melatonin values (± SEM) and subjective sleepiness ratings are displayed in the lower panel for healthy young (HY, green filled circles; n=8), young depressed (MDD, black filled circles; n=9), and healthy older (HO, red open circles; n=8) volunteers. Half-hourly values for both variables were binned in 1.25-h segments across the 40-h protocol. Significant post-hoc comparisons of the interaction of the factors group*session are indicated at the bottom line of the top panel (open circle = HY > HO; p at least < 0.05; post-hoc analysis based on LSMEANS statement with Tukey-Kramer adjustment of p values). MDD were significantly more sleepy during the entire protocol compared to HY and HO (indicated with Asterisks in the lower panel, p < 0.01, mixed-model 2-way rAnova). Gray bars indicate schematically the scheduled nap episodes.*

Table 4.4: Melatonin timing, mean secretion and phase angles for each group

variable	mean value ± SD			t-test
	young depressed women (MDD	healthy young women (HY)	healthy older women (HO)	p
melatonin upward mean crossing time	22.15 ± 1.44	22.19 ± 1.39	21.83 ± 1.51	-
melatonin downward mean crossing time	8.47 ± 1.50	8.01 ± 0.99	7.11 ± 1.67	-
midpoint of melatonin peak (h)	3.31 ± 1.33	3.10 ± 1.09	2.47 ± 1.57	-
mean duration of melatonin secretion *	10.32 ± 1.25	9.82 ± 1.03	9.29 ± 0.55	0.05 (MDD > HO
mean melatonin secretion (pg/ml) *	18.43 ± 10.01	25.83 ± 14.93	10.69 ± 6.20	0.05 (HY > HO)
mean bedtime (h; clock time)	23.89 ± 0.96	23.61 ± 1.35	23.41 ± 0.86	-
phase angle 1 (h; bedtime - upward mean crossing time)	1.74 ± 1.34	1.43 ± 0.70	1.58 ± 1.28	-
phase angle 2 (h; [bedtime - downward mean crossing time])	8.58 ± 1.54	8.40 ± 1.35	7.71 ± 1.33	-

*The table shows different characteristics of the melatonin secretion for each group (mean ± SD). Furthermore, the phase angle between external and internal circadian phase expressed as difference between habitual bedtime and upward or downward melatonin mean crossing time respectively is mentioned. MDD = young depressed (n=9), HY = healthy young (n=8), HO = healthy older (n=8) women. Indicated p values are derived from a t-test for independent samples. *With reference to secretion between upward and downward mean crossing time.*

4.4 Discussion

Our data indicate clear alterations in homeostatic sleep pressure in young depressed compared to healthy young and older women as manifested by significantly higher frontal EEG delta activity (i.e. marker of sleep homeostasis) across the baseline, recovery nights and the 10 nap episodes along with more stage 4 sleep and significantly higher subjective sleepiness levels. These changes occurred without significantly affecting sleep architecture in depressed women. These results were rather unexpected since a reduction in EEG delta activity and slow-wave sleep along with more REM sleep, particularly at the beginning of the night was previously reported in many studies [185, 192, 194]. Furthermore, comparisons with the healthy older women showed a similar reduction in nighttime melatonin levels in the depressed women and a similar increase in sleepiness levels, particularly at the end of the nap protocol in both the older healthy and young depressed compared to the young healthy women.

Sleep stages and homeostatic sleep regulation

Young depressed women showed no significant differences in sleep architecture during the

baseline and recovery nights compared to healthy young women except for less stage 1 sleep (most likely but not statistically significant at the cost of more stage 4 sleep). Less stage 1 sleep indicates a faster transition to higher NREM sleep stages in our depressed cohort. These results contrast commonly reported sleep pattern alterations associated with major depression such as reduced SWS and shortened REM sleep latencies as well as increased REM sleep [204, 301, 263]. However, it has been shown that changes in sleep architecture in depression depend on sex, age, depression subtype, and the severity of the illness and can therefore not been generalized to all individuals with depression. Furthermore, our recruitment criteria for depressed women excluded individuals with major sleep problems such as decreased sleep efficiency or prolonged latency to sleep onset in order to investigate rather the influence of depression per se on sleep regulation and to allow for a more stringent comparison with the non-sleep disturbed healthy control women.

Our cohort of healthy young and older women originated from previous studies encompassing healthy older women and men. We could confirm age-related differences in sleep architecture across the baseline and recovery nights such as less total sleep time, less stage 4 sleep, and a lower sleep efficiency between healthy young and older women [197]. We now extend these findings by showing that young depressed women differ similarly as young healthy women from healthy older women with reference to the aforementioned sleep parameters. However, unexpectedly young depressed women had significantly more stage 4 sleep across the 10 nap epidsodes relative to baseline night values compared to the other two groups.

Two EEG markers of homeostatic sleep pressure have been described: frontal delta activity (0.75-4.5 Hz) during NREM sleep and low frequency EEG activity in frontal brain regions during wakefulness which increases during wakefulness and parallels the sleep-wake dependent increase of delta activity during sleep [302]. In all three groups we observed a significant relative decline of EEG delta activity during the first NREM-REM sleep cycle in the recovery night. This result not only confirms previous findings on the alleviative effect of napping on homeostatic sleep pressure during subsequent night sleep episodes [303, 304] but also extends them to females with major depression. Our data show that young depressed women had significantly higher absolute EEG delta activity compared to the other two groups across both baseline and the recovery nights in frontal brain regions, while relative EEG spectra values (recovery night spectra expressed as a percentage of respective baseline values; data not shown here) did not significantly differ. Moreover, young depressed volunteers exhibited significantly higher delta sleep EEG activity compared to healthy young during the naps occuring at the biological day. Hence, we conclude that the more pronounced increase in EEG delta activity in young women with MDD reflects higher homeostatic sleep pressure rather than an impairment in the homeostatic response to low sleep pressure conditions. Although this conclusion remains to be underlined by the analysis of the low frequency EEG activity during wakefulness the significantly higher subjective sleepiness ratings of young depressed women compared to both other groups raises further evidence for our conclusion as a close correlation between these objective and subjective sleepiness parameters have been reported[305]. However, it has been suggested recently that slow-wave sleep and EEG

slow-wave activity may play a crucial role in the treatment of depression as selective EEG slow-wave deprivation without changing habitual sleep duration has been shown to improve mood in depressed volunteers remarkably and has been therefore associated with the known antidepressant effect of a night's sleep deprivation [306]. Moreover, there is also evidence that an enhancement of slow-wave sleep potentially decreases positive mood in depressed patients while having a contrary effect in healthy controls [307].

From a neural perspective different aspects may contribute to higher homeostatic sleep pressure in depression. Recently, the neural correlates of rumination (recursive self-focused thinking) in depression have been identified through a fMRI study [308]. Depressed individuals showed higher activation in the medial and dorsolateral prefrontal cortex and in limbic structures during rumination than healthy controls. Although it is not clear yet whether the activation of the dorsolateral prefrontal cortex reflects impairment of neural regulatory mechanisms or neural recruitment towards cognitive demand one may still speculate that during rumination additional synaptic potentiation and hence changes in cortical plasticity may occur. According to the synaptic homeostatis hypothesis plastic changes during wakefulness leading to an increase of synaptic strength are strongly correlated to the amount of slow-wave activity during subsequent sleep and this particularly, when synaptic potentiation occurs in cortical areas [103]. Within the framework of this hypothesis slow-wave sleep serves to downscale synaptic strength to an energetically sustainable level. Hence, taken together the cortical correlates of rumination, the dependency of cortical activation during wakefulness and slow-wave sleep as well as our results of higher frontal delta activity in depressed young women we argue that higher rumination levels in our depressed individuals compared to the other two groups may have been present. One limitation of these speculation on the neural correlates of higher homeostatic sleep pressure in young moderately depressed women without major sleep disturbances is that we did not measure the rumination degree in our participants (healthy and depressed). Furthermore, it remains to be studied whether rumination in depression may induce higher synaptic potentiation such as cognitive tasks and subsequently lead to higher NREM sleep slow-wave activity.

From a molecular perspective, homeostatic sleep regulation has been suggested to be associated with the nucleoside adenosine which may play a role as endogenous somnogen [86, 87]. This assumption is based on the observations that adenosine levels rise throughout the brain during prolonged wakefulness and decrease during sleep [88, 89] as well as that caffeine being an adenosine antagonist increases alertness and decreases sleepiness [90]. Furthermore, it has been shown that people with elevated adenosine levels due to a functional polymorphism in adenosine deaminase show increased levels of slow-wave sleep and less nocturnal awakenings [309]. Hence, the increased level of slow-wave sleep and EEG slow-wave activity in our cohort of young depressed women could be a result of increased adenosine levels (and vice versa for healthy older women). However, this remains to be elucidated, also because studies on adenosine deaminase levels in depression have shown both, increased and decreased levels [310, 311].

The dynamics of EEG slow-wave activity across the baseline and recovery nights were not different between the three groups although the slope of the exponential decay function appeared to be higher in young depressed subjects during the recovery night. However, healthy young differed from the other two groups with respect to the ultradian SWA regulation across NREM-REM cycles during the recovery night, such that they exhibited a significant intrasleep rebound of EEG slow-wave activity during the NREM sleep episode 3 compared to prior NREM sleep episodes which was not present in young depressed and healthy older women. The resurgence of EEG slow-wave activity during later parts of the sleep episodes have been observed either after selective slow-wave sleep deprivation or during extended sleep episodes of up to 14.9 hours [312, 85]. While in the former case slow-wave sleep rebound occurred because homeostatic sleep pressure was still present from prior wakefulness, the intrasleep rebound in connection with prolonged sleep duration has been suggested to represent a circasemidian sleep-dependent rhythm [313, 314]. As neither of these explanations serves to explain the observed SWA intrasleep rebound in young healthy women in our study it remains unclear whether it reflects a sleep-wake dependent (homeostatic) process or if, due to the low sleep pressure conditions, it is a response to a sleep promoting signal of the circadian pacemaker around the sleep maintenance zone.

Circadian sleep-wake modulation

The distribution of the 10 nap episodes over a time period of 40 hours allowed sleep to occur at different circadian phases thus resulting in significantly varying sleep efficiency and total sleep time during the naps. This circadian control of sleep duration is in accordance with the two-process model of sleep regulation and previous study findings [315, 53, 72].

While it has been demonstrated that EEG slow-wave activity is practically uniquely dependent on the prior history of sleep and wakefulness, the modulation of sleep spindles (12-15 Hz) is mainly under circadian control with high values during the early subjective night and low values in the early morning [55]. Furthermore, a melatonin-related shift of spindle frequency peaks has been shown in healthy young subjects such that during the biological day when melatonin secretion is low spindle peaks occur at higher frequencies than during the biological night [316, 317, 318]. This spindle modulation between the biological day and night was clearly reflected in healthy young women by both a significant low (12.25-13.75 Hz) and high (14.75-15.25 Hz) spindle peak difference. This circadian modulation of spindle frequencies was less pronounced in the young depressed women as differences between biological day and night were only significant in the high spindle frequencies (14.75-15.25 Hz). In healthy older women, no significant differences in this circadian modulation was observed, they were virtually absent. Correspondingly, the relative nocturnal increase in low spindle frequencies was significantly higher in healthy young compared to young depressed (13.5-13.75 Hz) and compared to healthy older women (12-13.75 Hz). Furthermore, the nocturnal decrease in the higher spindle frequency range (15 Hz) was not only significantly less intense in older healthy compared to healthy young women as described by Münch et al. [278]

but also significantly less pronounced compared to young depressed women. The lack of a circadian modulation of sleep spindles between the biological night and day together with significant lower values of mean melatonin secretion in healthy older compared to healthy young women during the biological night has been reported to add evidence for a weaker circadian signal in sleep-wake regulation [278]. Our young depressed subjects thereby appear to display some of these aspects observed in healthy aging, but to a lesser degree as the circadian modulation of spindle peaks seemed to be partially intact and melatonin secretion only show a tendency to lower values compared to age-matched healthy individuals.

The internal coincidence hypothesis states that sleeping at the wrong circadian phase is depressogenic [217]. While this hypothesis was based on the assumption that the circadian clock is advanced compared to sleep timing more recent studies rather suggested a delayed circadian phase of dim-light melatonin onset relative to sleep timing to be involved in the modulation of depressive symptomatology [319]. Furthermore, a recent study observed no circadian misalignment of the sleep-wake timing (mid-point of sleep) and the endogenous circadian pacemaker as measured by the core body temperature minimum and dim-light melatonin onset in depressive patients compared to healthy individuals [320]. In accordance with the latter we found no phase advance or delay of the endogenous rhythm of melatonin (upward and downward mean crossing times) relative to habitual sleep time in young depressed women compared to healthy young and older females. Mean bed- and wake-up times also did not differ between the three groups. Young women with depression showed only a significant longer melatonin secretion duration compared to healthy older women indicating a different circadian coding for the duration of the biological night. Taken together, the observed changes in the sleep pattern of young depressed women compared to healthy women were most likely not due to a circadian misalignment of sleep-wake timing with respect to the endogenous circadian pacemaker.

Conclusions

Our results provide strong evidence for higher homeostatic sleep pressure in young moderately depressed women. Hence, our data on slow-wave sleep and EEG slow-wave activity do not support a homeostatic deficiency in young depressed women without sleep problems as proposed by the S-deficiency hypothesis for depressed patients with sleep problems [112, 113]. Our data clearly show dissimilar homeostatic sleep-wake regulation in depression than in healthy aging. Furthermore, the observed sleep-wake alterations in depression could not be related to a circadian misalignment of sleep-wake timing and the endogenous pacemaker. Although our data could not fully highlight alterations of circadian processes involved in sleep-wake regulation a less pronounced modulation of the spindle frequency peaks between biological day and night and the reduced nighttime melatonin secretion point towards changes in the strength of the circadian output signal in depression. Along these lines, higher levels of sleepiness and EEG slow-wave activity during the biological day could

reflect a dysbalance of the opponent interaction between the circadian and homeostatic processes in depression, such that a weaker circadian output signal led to an overexpression of sleep-wake homeostatic influences, which was further boosted by increased rumination by the depressed women leading to an increase of synaptic strength, which has been shown to correlate to the amount of EEG slow-wave activity during subsequent sleep.

Thus, our study provides first insights into homeostatic and circadian sleep-wake regulation in young women with major depression under low sleep pressure and the unmasking conditions of a constant routine protocol.

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Supplemental material

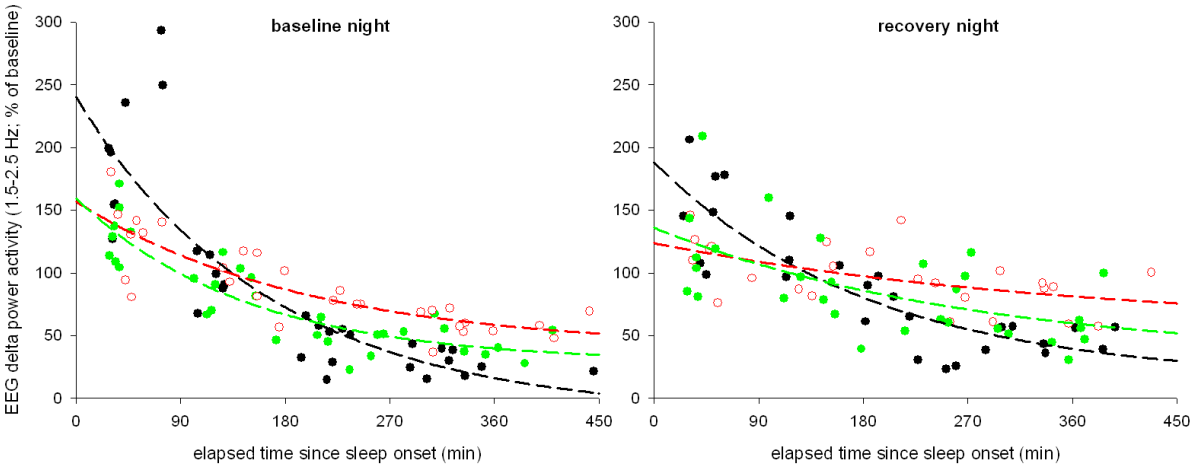

Figure S1: Fitted exponential decay of delta power activity during baseline and recovery nights

The figure displays the fitted exponential decay function [$delta_t = delta_\infty + delta_0 * e(-rt)$] to relative EEG delta power (1.5-2.5 Hz; percentage of baseline night) across all NREM sleep episodes for collapsed frontal derivations during baseline (left hand panel) and recovery nights (right hand panel). Young depressed women = black filled circles (n=8); healthy young women = green filled circles (n = 8); healthy older women = red open circles (n = 8).

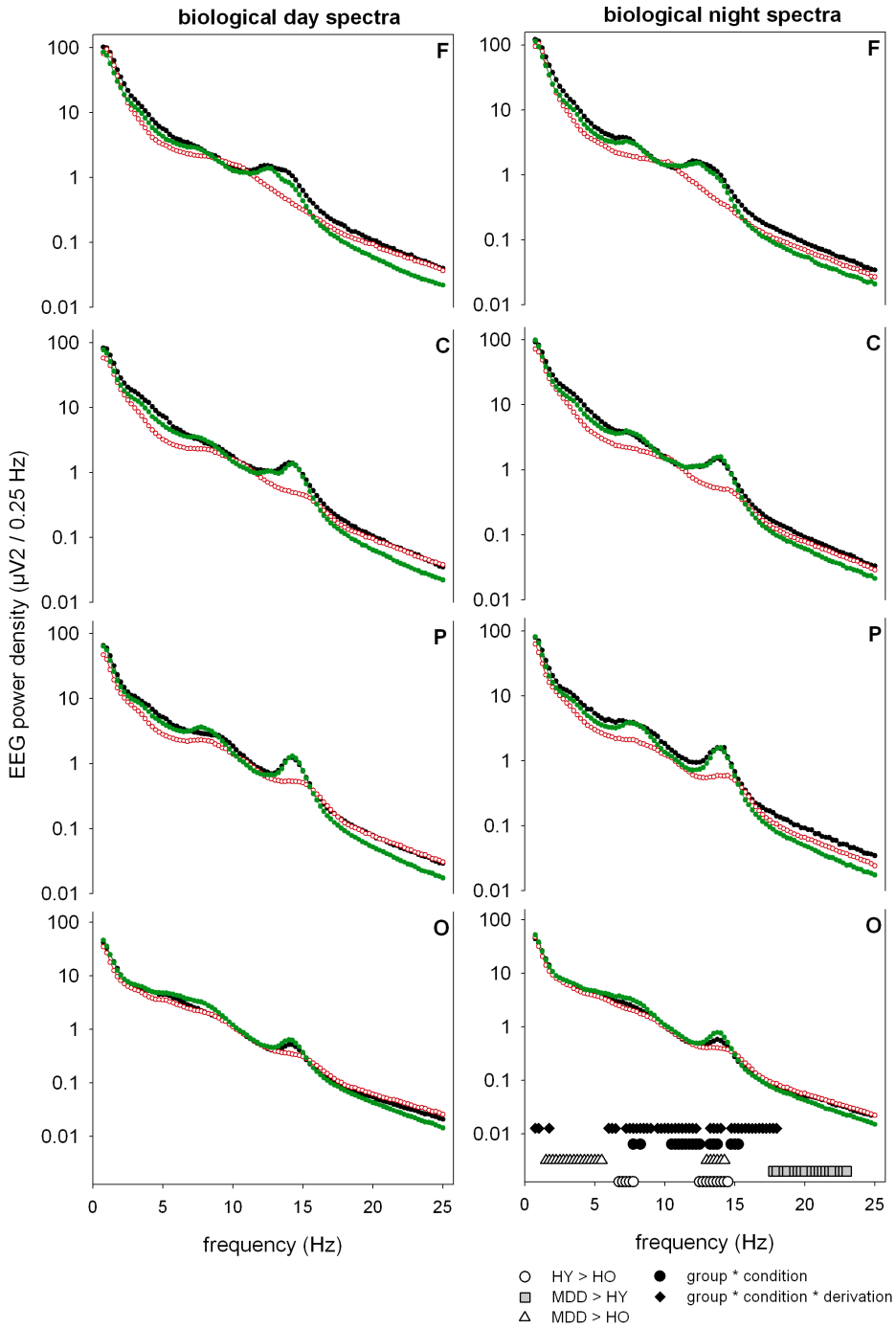

Figure S2: EEG power spectra for the nap sleep episodes during the biological night and day

*Absolute biological day and biological night EEG sleep spectra are derived from stage 2 nap sleep episodes. Spectra are shown in the frequency range 0.75-25 Hz for lateral and central collapsed F, C, P, and O derivations for young depressed (MDD; black filled circles; n=9), healthy young (HY; green filled circles; n=8), and healthy older (HO; red open circles; n=8) women. Post-hoc analysis on significant differences between the groups, significant interations between biological day and night and the groups, and significant interactions of the factors group*condition*derivation are indicated near the abscissa (p at least < 0.05; LSMEANS statement, Tukey-Kramer adjusted).*

5 Concluding and prospective remarks

Homeostatic and circadian aspects of sleep-wake regulation in women with respect to their sleep phase preference and developmental stage as well as the influence of major depression have been investigated in this thesis.

Sleep-wake regulation during female adolescence

In the first part of the thesis as presented in chapter 2, we aimed at establishing a relationship between menarche, as a developmental marker, and the apparent changes in sleep timing during adolescence. Our results provide evidence that a clear shift in sleep-wake cycles is temporally linked to menarche. Furthermore, it heralds the beginning of adult-like sleep-wake behaviour in women and can thus be used as a (chrono)biological marker for the onset of adulthood. Interestingly, the range of the attained chronological age at 5 years after menarche (16.5-18.5 years) coincides with the mean age where final body size is reached in women (16-19 years). Thus, apart from the already suggested mediation of the pubertal drop in melatonin levels by physical growth there is evidence for an additional relationship between physical growth spurt and the circadian system at the very end of reaching final body size and physical maturation, respectively.

Our data do not allow differentiating between social and biological influences on sleep time preferences. Increased leisure activities and rather early school times strongly influence sleep and wake behavior in adolescents. However, our results indicate that the accumulated sleep dept during school times may not fully explain the sleep phase delay. We found strong evidence for a circadian misalignment in adolescents as they experience a so-called social jet-lag (delayed mid-point of sleep during free days) accounting for up to three hours at the nadir of delayed shift in phase preference 5 years after menarche while the difference of sleep duration between week and free days was only about 1.2 hours. This result is of particular importance since circadian misalignment of sleep-wake timing and the circadian pacemaker may lead to impaired alertness and cognitive performance during wakefulness as well as to sleep disorders such as insomnia. Particularly the latter has been shown to be an independent risk factor for the development of major depression [321]. In order to prevent sleep and mood disorders during puberty and in particular adolescence later school beginning in the morning has been suggested by different authors. However, given the fact that sleep phase preference during adolescence is a dynamic process as shown by our study, the extent of school time shifts per day and in particular over how many years have to be

considered carefully. Ideally, they should be equally dynamic as the sleep time developments in adolescents.

Based on the two-process model of sleep regulation and empirical data a model for the sleep phase delay in adolescents has been developed which links developmental changes to the involved homeostatic as well as circadian processes [114]. According to this model a higher restistance to sleep pressure as indexed by a slower accumulation of EEG slow-wave activity and a significant longer endogenous circadian period (mean period length τ = 24.27 hours) than usually observed in adults (mean period length τ =24.12 hours) together with increased sensitivity to the phase delaying effect of light are responsible for the sleep-wake phase delay in adolescents [322, 323, 114, 324]. Importantly, there is evidence for the involvement of gonadal hormones to be necessary for the development of the phase delay [325, 326]. Thus, the hormonal and physical changes during puberty and adolescence may therefore not only be interpreted as physical maturation but also as developmental period of the homeostatic and circadian regulation of the sleep-wake cycle. In contrast to this period of circadian phase delaying during puberty and adolescence the anew alignment of circadian phase at the transition to adulthood is less well studied. Our data give first evidence that an additional physiological marker linked to physical maturation is most likely also involved in the transition to adulthood.

Our study cannot explain the mechanism responsible for the switch to the advance in sleep timing preference which occurs 5 years after menarche, but it provides an individual and hence rather precise timepoint to start further investigations. Ideally, in order to follow the sleep phase changes during adolescence more closely longitudinal studies starting between the onset and 5 years after menarche and lasting until around 9 years thereafter (when the circadian misalignment starts to be significantly decreased) should be initiated. Furthermore, at it has been shown that the circadian pacemaker contributes to individual sleep timing and duration, studies on sleep-wake phase changes in adolescents would need to encompass circadian markers such as melatonin. Moreover, subjective sleepiness measures or, if performed as laboratory studies, objective measures of homeostatic sleep pressure by EEG slow-wave activity could further serve to highlight aspects of the homeostatic sleep drive. Moreover, with reference to the afore mentioned phase delay model, it would be interesting to investigate whether changes in endogenous circadian period are involved in the advancing of circadian phase after 5 years after menarche. Apart from analysing circadian clock gene expression on the basis of fibroblast cells [327] which requires tissue biopsy sampling a promising new and less invasive method requiring hair follicle cells from the head [328] would definitively facilitate such attempts.

Homeostatic and circadian sleep-wake regulation in young women with major depression

We studied homeostatic and circadian sleep-wake regulation in young moderately depressed women under high and low sleep pressure in constant routine conditions. These conditions stand out by a stringent control of environmental and behavioural conditions which may mask the expression of the endogenous rhythms generated by the circadian pacemaker and are thus a necessary prerequisite for the study of homeostatic and circadian aspects as described in chapter 1 of this thesis. Apart from comparing young depressed with age-matched controls, the aim of this second part of the thesis was also to test the homeostatic (S-)deficiency hypothesis in depression [113] as well as the early notion that sleep in depression resembles sleep in older individuals (i.e. premature aging with respect to sleep) as described in chapter 3. Despite of being a promising model, which may explain chronotherapeutic interventions such as sleep deprivation in depression, only very few studies have tested the S-deficiency hypothesis so far. This requires manipulation of wakefulness prior to sleep and subsequent analysis of low frequency EEG response as electrophysiological marker of homeostatic sleep pressure. Hence, our studies as presented in chapter 3 and 4 provide first insights into sleep-wake regulation in depression in response to different temporal manipulations of sleep and wakefulness under the unmasking conditions of a constant routine protocol.

The results in our young unipolar depressed women show higher homeostatic sleep pressure as indexed by higher EEG slow-wave activity (SWA) compared to healthy controls. Importantly, this enhanced sleep drive and amount of SWA respectively was present irrespective of the applied state manipulations of the sleep-wake cycle (e.g. sleep deprivation vs sleep staturation). Hence, our study raises not only evidence for alteration in the homeostatic sleep regulation in female depression but also points towards a trait-dependency of this alteration. Final proof of this assumption would require studying the same depressed women after successful treatment for their depression, thus in a non-depressed state.

Our conclusion for higher homeostatic sleep pressure in young depressed women was further underlined by higher low frequency EEG values during extended wakefulness (not presented in this thesis). Furthermore, young depressed individuals exhibited significant higher subjective sleepiness under low sleep pressure conditions as presented in chapter 4. Interestingly, subjective sleepiness between young healthy and depressed volunteers appeared rather similar during sustained wakefulness (chapter 3) which is not supportive of our conclusion of higher sleep drive in depression at first sight. However, the comparison of the sleepiness ratings during sustained wakefulness in relation to baseline sleepiness values as presented in Fig. 5.0.1 shows that young depressed individuals were as sleepy after 40 hours of wakefulness as before the baseline night (= 100%) which stands in clear contrast to the higher relative subjective sleepiness of young healthy volunteers (> 125% of the baseline sleepiness value) just before recovery sleep. Hence, prolonged wakefulness of 40 hours did not affect sleepiness levels of young depressed women more as a normal day (i.e. 16 hours). This

finding may reflect a habituation to a life under high homeostatic sleep pressure in depression. This assumption is further supported by the fact that significant differences between subjective sleepiness values of the high and low sleep pressure conditions were only present in young healthy but not in depressed women (see Fig. 5.0.1).

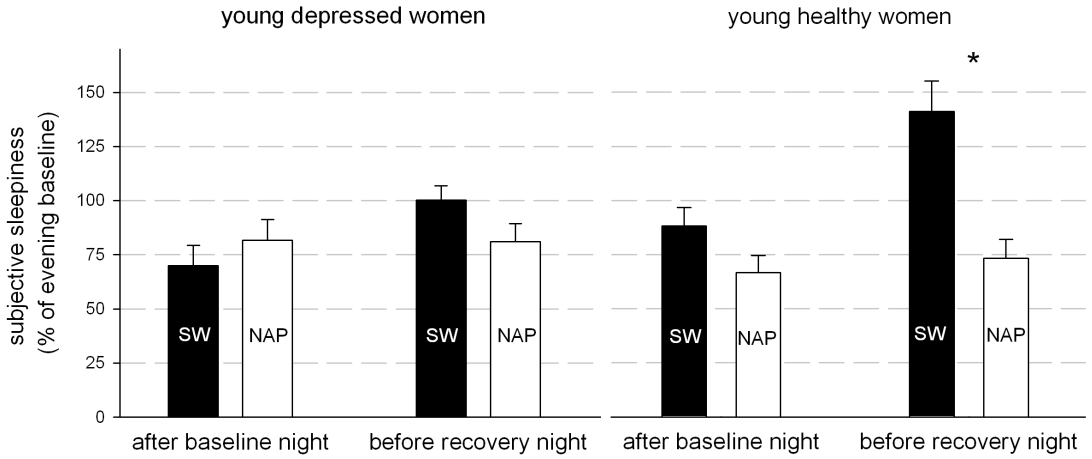

Figure 5.0.1: Relative subjective sleepiness at the beginning and the end of the low and high sleep pressure conditions in young depressed and healthy women

Relative subjective sleepiness is shown for the first and the last ratings (half-hourly ratings binned in 3.75-h segments over the 40-h protocol) between baseline and recovery nights during the sustained wakefulness (SW) and the nap protocol (mean ± SEM). Subjective sleepiness ratings are depicted as percentage of the ratings before baseline night. Mean sleepiness values of young healthy but not of depressed volunteers differed significantly between the two sleep pressure conditions. Moreover, subjective sleepiness ratings of young healthy women were significantly higher during sustained wakefulness compared to the nap condition at the end of the 40-h protocol as indicated by asterisks (mixed-model 2-way rAnova performed for each group separately, post-hoc analysis based on LSMEANS statement).

Different aspects may contribute to higher homeostatic sleep pressure and EEG SWA in depression such as neural, chemical, and genetic processes. According to the synaptic homeostasis hypothesis the amount of EEG slow-wave activity during sleep, which represents synaptic downscaling, has been shown to be clearly correlated with the extent of cortical activation and thus synaptic potentiation during prior wakefulness [103]. Although not well-understood yet, rumination in depression, as one of the key factors for the onset and maintenance of the illness [329], may reflect higher cognitive demand and thus lead to additional cortical activation [308] and higher SWA as observed in our groups of young depressed women compared to the two healthy groups. From a chemical perspective, higher levels of adenosine, a nucleoside proposed to act as endogenous somnogen [86, 87], may entail higher SWA in depression. Beside these altered neural and chemical processes genetic alternations may also contribute to differences in slow-wave sleep (SWS) and slow-wave activity. Studies on clock genes have shown that alternations in PERIOD3 for instance influence individual differences in slow-wave activity and sleep phase preference in humans for example [330]. However, these neural, chemical or genetic alterations with reference to homeostatic sleep drive in depression remain to be elucidated in future studies.

Our results on higher sleep drive in our group of depressed women do not conform to the claims of the homeostatic deficiency hypothesis in depression [113]. However, our results cannot disproof the hypothesis since our young depressed women did not have major sleep disturbances, which was a sine qua non for the S-deficiency hypothesis. Hence, it remains to be assessed whether the hypothesis holds for depressed women with sleep disturbances. We could clearly show that depression does not equal premature aging with respect to sleep as noted by Gillin [195] as our data show a clear overexpression of homeostatic responsiveness in depression as measured by SWS and SWA in contrast to an attenuated homeostatic sleep response with age. Potential reasons for a weaker homeostatic sleep response may be due to an age-related decline in adenosine A1 receptors as recently shown in a human positron emission tomography (PET) study [331] or a reduced synaptic potentiation during wakefulness due to mild cognitive impairments typically observed in healthy aging which in turn results in less SWA during subsequent sleep [332].

In addition to homeostatic sleep alteration, results in chapter 3 and 4 also give evidence for circadian changes in depression. Depressed women exhibited lower melatonin secretion compared to healthy young under both sleep-wake manipulation schemes although this result was more pronounced in the group who followed the high sleep pressure protocol. There are conflicting results in the literature with respect to melatonin levels in depression. However, the commonly found deficiencies in serotonin in depression may explain reduced melatonin levels and support the view of depression as low melatonin syndrome [286, 333]. Moreover, some antidepressants encompassing not only neurotransmitter deficiencies but also acting as melatonin receptor agonist support the view of decreased melatonin levels in depression. The increase of melatonin appears to benefit the treatment of depression by an amelioration of associated sleep disturbances [334, 335].

We could not confirm commonly mentioned circadian phase misalignments between sleep timing and endogenous pacemaker in depression [217, 319]. This result is somehow limited because we included only intermediate chronotypes in our study who are probably at the lowest risk to develop abnormalities between external and internal clock. However, the convergence on intermediate chronotypes would not interfere with the occurrence of possible endogenous circadian phase misalignments (i.e. internal desynchronisation) between the cortisol and melatonin rhythm for example as it has been recently shown [221].

Our findings highlight the influence of depression per se on homeostatic and circadian sleep regulation in young moderately depressed women without major sleep disturbances as a specific endophenotype in depression because due to our selection criteria the illness was the only differentiating aspect between the young depressed and healthy control group. In this sense, our results on higher SWS and SWA as well as a weaker circadian output signal in young depressed women may also have clinical implications for the treatment of the illness. It has been shown recently that selective SWS and SWA deprivation lead to mood improvement [306] whereas enhancement potentially decreases positive mood in depression [307]. Hence, in contrast to total sleep deprivation which did not improve mood in our

cohort of depressed volunteers (see chapter 3) a selective decrease of SWS and SWA could serve as an effective therapeutic intervention in this endophenotype of depression. Thereby, in contrast to SWS and SWA deprivation the recently developed rumination-focused cognitive behaviour therapy (RFCBT) [336, 337] could benefit long-term amelioration of the homeostatic sleep drive according to aforementioned assumptions on the neural effects of rumination in connection with the synaptic homeostasis hypothesis. Apart from interventions on homeostatic sleep processes the treatment of young moderately depressed women could also encompass a strengthening of the circadian output signal by carefully timed bright light administration as a chronobiological therapy which has been shown to have beneficial effects on mood in non-seasonal major depression [338]. Thereby, mainly as a function of the administration timing bright light therapy may induce the suppression of melatonin, a shift of the melatonin rhythm but also increase melatonin amplitude as recently demonstrated in older depressed patients [339].

Our data on sleep regulation in depression disclose several interesting future research questions such as the assessment of the S-deficiency hypothesis in young moderately depressed women with sleep disturbances. An adequate test of this hypothesis would require equal stringent study conditions (constant routine conditions) as presented in chapter 3 and 4 in order to highlight unmasked circadian and homeostatic sleep regulation. Furthermore, the investigation of the correlation between rumination in depression and increased homeostatic sleep response would add important insights into the neural mechanisms in homeostatic sleep regulation in depression. Thereby, a study design could encompass baseline polysomnographic sleep measurements in the laboratory under constant routine conditions together with the assessment of the baseline rumination level by the Response Styles Questionnaire [329] in depressed study volunteers. Thereafter, as an ambulatory part, the aforementioned RFCB therapy could be applied over several sessions [337] accompanied by regular rumination level assessments followed by an anew laboratory polysomnographic sleep recording in order to assess the effect of the therapy on homeostatic sleep regulation. The effect of bright light therapy on the amelioration of the circadian and homeostatic sleep regulation in young moderately depressed women without major sleep disturbances could be studied in a similar study protocol. Both studies would certainly highlight important mechanisms of sleep regulation in depression as well as add invaluable support to the treatment of major depression.

Taken together, this thesis provides several new insights into circadian and homeostatic aspects in the sleep-wake cycle in women during maturation and in depression. We could establish an association between changes in circadian sleep phase preference during female adolescence and physiological maturation and we could demonstrate the existence of a trait dependent disbalance of the opponent interaction between circadian and homeostatic sleep regulation in young depressed women without the existence of obvious sleep disturbances.

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